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ERRATA

Page 75, paragraph 3, line 10, after sodium add silicate.

Page 76, small table, combine first column headings, Average daily losses, 180-day incubation, applying to both columns.

Page 79, paragraph 3, line 2, change index 10 to 3; delete footnote 10.

Page 108, paragraph 2, line 7, after reaction of insert all.

Page 149, table 4, legend, read Sclerotinia for sclerotinia.

Page 159, paragraph 3, line 1, read sulphite for sulphide.

Page 160, table 14, column heading 2 under Pentathionic acid, read 0.00068 N. for 0.0068 N.; table 14, line 6 under Pathogenic fungi tested, read Botrytis for Botrytus.

Page 197, paragraph 2, line 2, read have for has.

Page 234, paragraph 3, line 9, change This form to Thus, formation.

Page 344, figure 11, legend, line 4, read B. for D.; line 11, and page 345, line 1 and 2, substitute apparently have mistaken the gelatinous egg-containing matrix for the dead body of the female filled with developing eggs for apparently have mistaken the dead body of the female filled with developing eggs for the gelatinous egg-containing matrix.

Page 397, paragraph 2, line 2, read Neofabraea for Neofabreae.

Page 399, paragraph 4, lines 10 and 11, *substitute* agar, corn-meal, malt, Dox, apple, and beef agars *for* agar. Corn meal, malt, Dox, apple, and beef agars also were used.

Page 481, paragraph 4, line 1, read cavavaliae for canabaliae.

Page 497, figure 1, legend, lines 1 and 2, read Marquis for Marquiz.

Page 548, line 6, read 549 for 56.

Page 784, line 3, read facultative for faculative.

Page 14, line 22, Supplement to Number 9, read MacLachlan, J. D., for McLachlan, J. D.; line 56, read Miyabe, Kingo, for Miyaba, Kingo.

Page 817, table 1, legend, read data for date.

Page 822, line 14, read was for and.

Page 885, table 4, legend, read hypochlorite for hypochlorite; line 10, after cent insert of.

Page 888, paragraph 2, line 11, after acid insert (=3 per cent commercial HCl by volume).

Page 889, paragraph 4, line 2, change maximum to usual.

Page 896, table 11, column 5, change 86 to 96.

Page 938, line 5, read Celastrus for Celastris.

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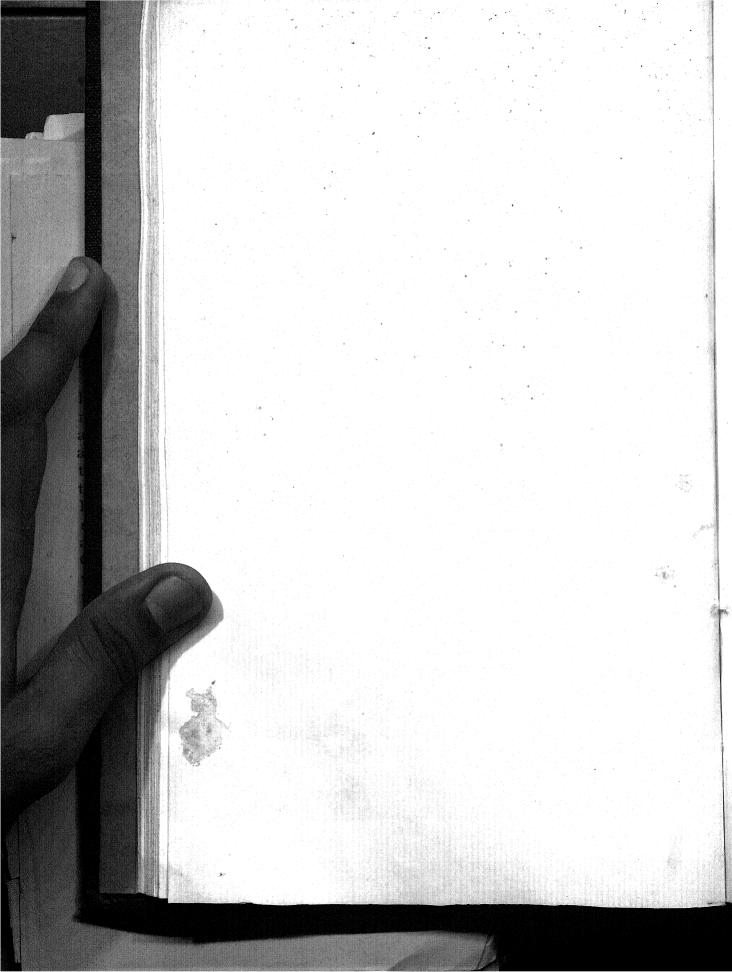
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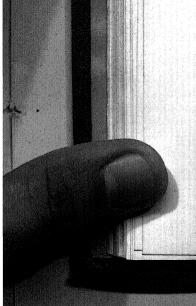
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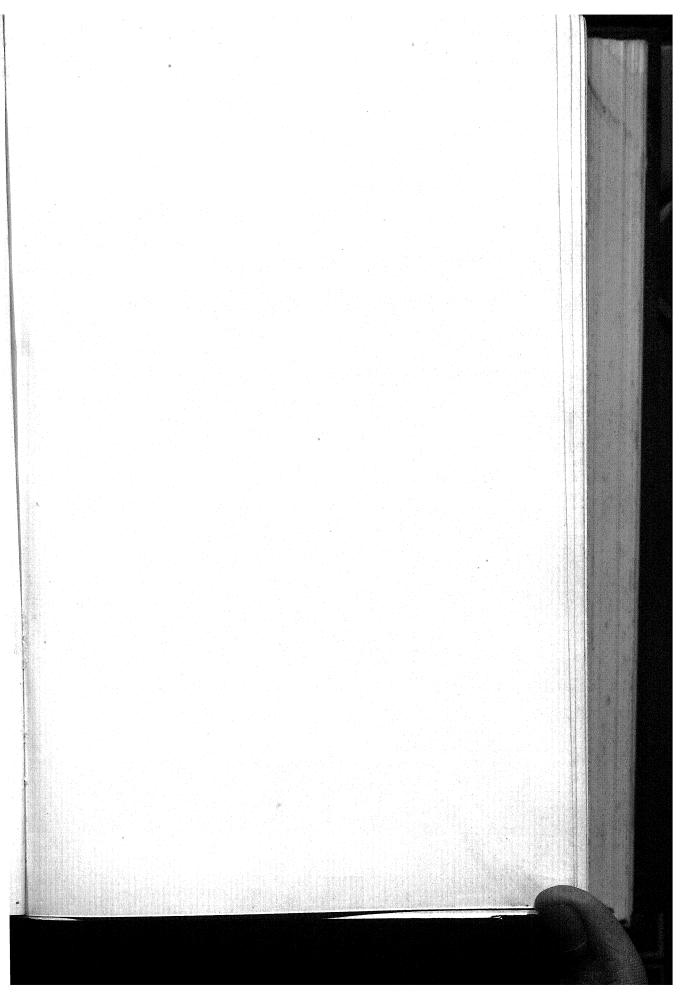
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Howard, N. O.	1916	1930
Jaczewski, A. (patron)	1921	1932
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3 Members who have died since the publication of the list in Phytopathology in October, 1925.





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Causes and control of damping off of tomato seedlings. L. J. Alexander, H. C. Young, and C. M. Kiger.

Two fungi, Pythium ultimum and Rhizoctonia solani Kühn (Corticium vagum B. & C. var. solani Burt), have been found to be the causes of damping off of tomato seedlings, in greenhouses. Inoculation experiments demonstrated that P. ultimum caused the major loss. The symptoms of the two diseases are similar but differ in that R. solani causes malformations of the cotyledons and produces a drier type lesion than P. ultimum. In soil-temperature and soil-moisture experiments P. ultimum was least destructive at 30° C. and was most destructive between 18° and 24° C. Dry soil was found unfavorable to the development of both diseases. Soaking tomato seed in any of several strengths of copper sulphate solution increased the percentage of emergence but, later, the seedlings damped off severely. In sterilized soil, to which pure cultures of either P. ultimum or R. solani had been added, excellent control of the disease was secured by disinfecting it with a formaldehyde dust. The dust was prepared by adsorbing 15 parts by weight of 40 per cent commercial formaldehyde on 85 parts of a carrier composed of 1 part of Kaolin and 2 parts of diatomaceous earth. The best control was secured when 42 grams of the dust was mixed with each square foot of seed bed soil, which was 2½ to 3 inches deep. Immediately after treating, seed was sown and the soil was thoroughly wetted. Several other chemical mixtures were tried but gave unsatisfactory control of damping off.

Canker of black walnut caused by a Nectaria sp. J. M. ASHCROFT.

A survey of West Virginia has located the canker of black walnut reported by Orton (Science 72: 142-143. 1930) in 21 counties of the State. This disease has been observed also in Pennsylvania and Virginia, and specimens have been received from Rhode Island, Wisconsin, and Ontario. Reports have been received of its presence in 6 other counties of West Virginia as well as in Tennessee and North Carolina.

A Nectria is almost invariably associated with the canker. Inoculations were made by inserting conidia from pure cultures of this organism into wounds made in the bark of old and young black-walnut trees. Out of 104 inoculations, made between February 18 and April 28, 79 per cent produced the symptoms of the disease. None of the controls showed such symptoms. The fungus has been repeatedly reisolated from the artificially induced cankers.

The characteristic ringed appearance of the canker results from a seasonal alternation of the dominance of the fungus growth with that of the host. The period of active growth of the fungus is late winter and early spring. All regions of the trunk are invaded by the fungus.

Studies of the fungus in culture and from natural sources reveal a close relationship with Nectria ditissima.

The resistance of certain varieties of grapes to Phymatotrichum root rot. Walter J. Bach and J. J. Taubenhaus.

Field inoculations at Substation No. 15 with *Phymatotrichum omnivorum* on grapes during 4 seasons have shown a difference in degree of susceptibility of varieties. Several varieties, which appear to be desirable as rootstocks, show a high degree of resistance. Approximately 104 varieties and 12 of the most promising rootstocks were included in the test. During the 1930 and 1931 seasons, in addition to the inoculation of the individual vines, cotton, a very susceptible host, was interplanted in the rows and also inoculated. The varieties that have withstood this test so far and also appear to be

vigorous enough for promising rootstocks are: Champanel, Mustang, Vitis Constancia, V. Salonis, Dog Ridge, V. Berlandieri, V. Champini, and Black Spanish. Excavations of grape root systems have revealed the presence of mycelium of the root-rot fungus on the roots near the points of inoculation on varieties that appear to be resistant. Since some varieties of grapes are very susceptible, the facteor of resistance must differ for varieties.

The pathogenicity of Bacterium translucens var. undulosum. R. H. Bamberg.

In addition to wheat, rye, barley, and speltz, Bacterium translucens var. undulosum infects oats, Hordeum jubatum, and Bromus inermis.

Cultures obtained from rye readily infected wheat seedlings and produced typical black-chaff lesions. Cultures obtained from barley with lesions similar to those previously described for *Bacterium translucens* also produced lesions on wheat seedlings typical of *Bact. translucens* var. *undulosum*.

The pathogenicity of cultures isolated from wheat from different localities varied considerably. Four variants arising as sectors differed from the parent cultures both culturally and in pathogenicity. Lesions appeared on inoculated wheat seedlings in 48 hours at about 24° C. (ordinary greenhouse temperature), but not until after 20 days at 10° C. In the field in 1931, wheat plants were readily infected early and late in the growing season but not during the heat of midsummer. Symptoms vary greatly with environmental conditions. Wheat plants in the boot stage, inoculated with Bacterium translucens var. undulosum, became infected on the leaves, awns, glumes, rachides, and necks. Infected areas on plants in the greenhouse became typically black, while most of those on plants kept at 10° and 20° C., under artificial light, remained yellowish and water-soaked in appearance.

(Cooperative investigations between the Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, and Minnesota Agricultural Experiment Station.)

→ Apple measles. Anthony Berg.

A dematiaceous fungus, isolated in the fall of 1930 from newly formed lesions of apple measles on water sprouts of the season's growth, proved to be the cause of a form of measles that has been very destructive in a large orchard in West Virginia. The fungus grows rather slowly on artificial media, which may account for the failure in previous isolation attempts. Oatmeal and malt-extract agar seem to be best suited to the growth and sporulation of the fungus. On the host, spores are produced apically on stiff, almost seta-like, dark brown, septate conidiophores that arise singly or in loose aggregates from a few pseudoparenchymatous cells on the surface of the dead epidermis. The spores are clavate to obclavate; usually 3-septate or more, olivaceous to almost hyaline. Germination in water is only from basal and apical cells by means of straight, narrow, slow-growing, unbranched germ tubes. Sporulation was found to be most abundant in nature during July and early August, corresponding closely with the period of maximum infection as checked in the field for 2 seasons. In the orchard where the disease was studied Red Astrachan, Maiden Blush, Rome, and Grimes were found to be most susceptible, while Transparent, York, Gano, and Chenango remained free from the disease.

Crown-gall-like hypertrophies on a conifer. J. G. Brown.

A cypress tree, Cupressus arizonica, on the campus of the University of Arizona, was apparently dying from the attack of bark beetles. It was found to have numerous

root galls varying in size from that of a pea to galls several inches in diameter. From these galls a bacterium was isolated, which grew on Patel's medium and caused small galls when inoculated into the tissues of *Ricinus communis*. From the galls on the latter the bacterium was again isolated.

Is Bacterium tumefaciens a mutant or one of the pleomorphic forms of Bacillus radiobacter? Nellie A. Brown and Lewis T. Leonard.

The frequent appearance of nonvirulent colonies of Bacterium tumefaciens on plates made from known crown-gall tissue; the colony and various cultural resemblances between Bact. tumefaciens and Bacilius radiobacter; the appearance of tumor-like outgrowths on various hosts from which radiobacter was isolated and not Bact. tumefaciens; and more especially the appearance of galls \(\frac{2}{3}\) to 1 cm. across in less than 2 weeks on non-inoculated cut stems of cowpeas, kept in a moist chamber from which radiobacter was isolated, have contributed to the idea that Bact. tumefaciens may be either a mutant, a physiologic form, or one of the pleomorphic forms of B. radiobacter.

Bacillus radiobacter was isolated from the cowpea-stem tumors, and various host plants, including cowpeas, were inoculated with it. The cowpea plants were placed under varied conditions of temperature and moisture, but no outgrowths similar to the original ones developed. Filtrates of these cowpea-radiobacter cultures through Chamberland and Berkefeld filters were used alone for inoculations, also filtrates added to cowpea-radiobacter cultures a dissociate form of cowpea radiobacter was used alone for inoculations, also in combination with cultures, but no outgrowths developed. Following other simpler manipulatious, one strain produced definite small tumors, 5 to 6 mm. across, on daisy stems.

The effect of leaf rust, Puccinia triticina, on the composition and yield of winter wheats in 1931. R. M. CALDWELL, H. R. KRAYBILL, J. T. SULLIVAN, and L. E. COMPTON.

Yield data and chemical analyses of the plants and grain were secured from replicated plots of severely leaf-rusted and nearly rust-free winter wheats of 8 varieties grown at La Fayette, Ind., in 1931. Nearly rust-free control plants for the comparison were secured by frequent sulphur dusting. Other diseases were almost entirely absent. Separate analyses were made of the vegetative portions and kernels at the early-milk, late-milk, and dough stages and of the mature grain. The rusted plots consistently yielded a lower protein and much less vitreous grain than did the control plots, and, in Fulhard, a hard wheat, a greatly increased percentage of "yellow-berry" kernels. Conversely, the vegetative portions of rusted plants were higher in total nitrogen. Reducing sugars and sucrose were consistently lower in the vegetative portions of the rusted plants. Starch followed the same trend. Significant reductions in yield of grain and straw, test weight per bushel, weight per 1,000 kernels, and number of kernels per head were found in the rusted plots. In Michigan Amber, a susceptible variety, where 4 different degrees of rust severity were secured by variations in dusting procedure, the trends mentioned above were evidenced approximately in proportion to the rust severity.

(Cooperative investigations between the Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, and the Purdue University (Indiana) Agricultural Experiment Station.)

Appressorium formation and penetration by leaf rust of wheat, Puccinia triticina, in relation to stomatal aperture. R. M. CALDWELL and G. M. STONE.

By a method of stripping the epidermis from wheat-seedling leaves inoculated with leaf rust, and fixing, staining, and mounting it in absolute alcohol, it has been possible

to study directly the relation of the stomatal aperture at the time of penetration to the entrance of leaf rust into its host. Appressoria, substomatal vesicles, and infection hyphae are clearly evident in such preparations. Closed stomata offer no impediment to penetration of wheat seedlings by this rust. These studies indicate, rather, that the formation of an appressorium over an open stoma stimulates it to close tightly prior to penetration by the rust. This response of the guard cells would make penetration impossible. These observations on epidermis strips have been checked by similar findings on the living plants. Often a small stomatal slit is evident between the appressorium and the substomatal vesicle, apparently resulting from the penetration tube pushing between the guard cells. Inoculations with leaf rust at different temperatures had no effect in altering this relation to stomatal aperture.

Uromyces fallens, when inoculated onto wheat leaves, penetrated abundantly and behaved identically with Puccinia triticina in its relation to the stomata.

(Cooperative investigations between the Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, and the Purdue University (Indiana) Agricultural Experiment Station.)

Cultural races of Pestalozzia funerea and the production of variants resembling Monochaetia. CLYDE CHRISTENSEN.

Fifteen races of Pestalozzia funerea were obtained by isolating 150 individual conidia from acervuli borne on the needles of long-leaf pine, Pinus palustris. These races were distinguished from each other by the following cultural characters: rate of growth; amount of surface and aerial mycelium; color, topography, and zonation of the colonies; and abundance, distribution, and size of acervuli, and time required for their production. In addition, the spores of the different races varied in size, color, shape, and in length and number of setae. Ten variants, differing from their parents and each other in the characters listed above, arose in the form of sectors in cultures of the races isolated from pine needles. Races conforming to the description of Monochaetia were obtained from conidia isolated from pine needles and arose also as variants in monoconidial cultures that normally produced spores bearing 3 to 5 setae. Seven species of conifers were inoculated with spores of the different races, but none were parasitic under the conditions of the experiment.

Physiologic specialization in Helminthosporium gramineum. J. J. Christensen and T. W. Graham.

The barley-stripe organism, Helminthosporium gramineum, consists of numerous cultural and parasitic races. Eighty-four of 226 monosporous cultures, isolated from 76 different collections, were culturally distinct. Forty-nine of these were obtained from Minnesota and the rest from different regions in the United States, Canada, and Germany. With 2 possible exceptions, a distinct cultural race was secured from each collection. Occasionally 2 different races were isolated from a portion of a diseased leaf. The pathogenicity of 75 races was tested on several varieties of barley. The percentage of infection ranged from 0 to 90, some races being very virulent, some moderately so, and others relatively weak. In general, those barley varieties usually susceptible under field conditions were heavily attacked by a large number of races when artificially inoculated. However, some varieties were susceptible to some races but immune from or highly resistant to others. Symptoms vary with the variety and the parasitic race of the pathogen.

Control of powdery mildew of snap beans. HAROLD T. COOK.

Experiments on the control of the powdery mildew of snap beans were conducted at the Virginia Truck Experiment Station in the fall of 1931. Twelve different spray and dust fungicides were tested and, listed in the order of their efficiency, are lime-sulphur spray, Kolofog, Kolodust, sulphur-lime dust, dry lime-sulphur spray, copper-lime dust, Sulfocide, calcium mono-sulphide, Manganar dust, Bordeaux mixture, zinc Bordeaux, and Du Bay 1027TT. An experiment to determine the effect of the time of spraying and the number of applications on control gave the following results. The best control was obtained by spraying 4 times at weekly intervals beginning when the disease first appeared. Fewer sprays beginning at the same time or at later dates gave poorer control. Where series of 2 and 3 sprays were applied, the best results were obtained when the first application was made early.

Rotting of sugar-cane cuttings in Porto Rico. Melville T. Cook.

Thielaviopsis paradoxa is the major cause of the poor germination of sugar-cane seed cuttings in Porto Rico. It is a common and very abundant fungus and attacks many species of plants. It is primarily a wound parasite, penetrating the cut ends and destroying the cell walls of the parenchyma tissue. It causes characteristic reddish discolorations of the tissues, which eventually become black and rotten. The fibrovascular bundles, which persist after the other tissues are destroyed, can be pulled out of the decaying mass in brush-like bunches. The disease is most severe in the cooler months of the year and in the poorly drained soils. During the summer months it can be found in abundance in the cooler elevated regions.

Zonate eye spot on turf grasses. ARNOLD S. DAHL.

Zonate eye spot, caused by Helminthosporium giganteum H. and W., has been common on some turf grasses. The disease occurs on some strains of creeping bent (Agrostis palustris) and velvet bent (A. canina), which are propagated with stolons. It damages these grasses both in nursery rows and in turf. Under wet conditions the organisms spreads over the leaves of the plants so that entire leaves are killed and the turf is brown. There is a distinct difference in the susceptibility of the various strains of both creeping bent and velvet bent. The Virginia strain of creeping bent, widely grown in the Northern States, is extremely susceptible, while Washington and Metropolitan strains are resistant. Acme and Highland velvet bent are somewhat susceptible, while strain No. 14,276 is resistant. Satisfactory control of this disease was obtained by spraying the turf with solutions of ethyl mercury chloride and other experimental organic mercury compounds, such as ethyl mercury arsenate and phenol mercury acetate. These fungicides controlled the disease only when they were sprayed on the turf and left on the leaves. When the solutions were sprinkled on or when the turf was watered directly after the treatment, they were ineffective. Lime sulphur also checked the disease but was injurious to the grass. Chlorophenol mercury, bichloride of mercury, and calomel were used but did not prove satisfactory.

Relation of axillary-bud development to nodal smut infection in the corn plant. GLEN N. DAVIS.

Eight hundred plants were used to determine the relationship of axillary-bud development and the appearance of nodal smut boils; 400 of the plants were inoculated on June 13 and smut readings taken on August 21. On August 22, 100 plants, each, were injured by removing the ears, tops, or ears and tops, and 100 were held uninjured as checks. Four hundred uninoculated plants were treated likewise. Final smut

readings were taken on September 16. In the inoculated plants nodal infection in the uninjured checks increased 20.5 per cent. from August 21 to September 16. In the same period removal of tops resulted in a 29.0 per cent increase, removal of ears a 63 per cent increase, and removal of both tops and ears a 52.2 per cent increase over the checks. In the noninoculated plants nodal infection in the uninjured checks increased 33.3 per cent in the same period, removal of tops resulted in 43.7 per cent increase, removal of ears 56.2 per cent, and removal of both tops and ears 36.8 per cent increase. Seven out of 10 axillary buds from sweet corn inoculated 10 days previously showed smut mycelium when sectioned. One hundred and ten days after inoculation, 66 per cent of the apparently healthy buds from 10 of the plants showed small mature smut boils when held before a strong light.

A seedling-blight stage of onion bulb rot. GLEN N. DAVIS and CHAS. S. REDDY.

A number of onion growers have been compelled to rotate crops on their old land because of a seedling blight and bulb rot caused by a soil-borne Fusarium. Heavy losses are also sustained in onions grown for sets. In seedlings the tips whiten, die progressively downward and finally disappear. More mature, infected plants show the first symptoms as grayish white tips of the outer leaves. Usually only the upper half of the leaf falls over, but the upright lower half dies and becomes brownish gray. In early stages of seedling infection only 1 of the 3 or more roots, normally sparkling white, appears somewhat dull or lead color and, upon examination and isolation, is found to be parasitized. In older plants, the roots may be found in all stages of decay, finally collapsing. Bulbs rot both in the field and in storage. In plots at Clear Lake only 50 per cent of the original plants remained at harvest and many of these bulbs were diseased. In a large field at St. Ansgar, where maggots and smut were almost completely absent, 90 per cent of the original plants were lost, and the crop was a failure. The organism associated with this disease was sent to Dr. C. D. Sherbakoff for identification and was reported similar to, but not identical with, Fusarium zonatum form 1.

The genetics of the smut fungi. Sydney Dickinson.

In the covered smut of oats, Ustilago kollei, at least 7 pairs of cultural characters have been found to be segregated in definite ratios at the "reduction divisions." Two of these character pairs are segregated in only 1 ratio, another "color" is found to be segregated in 3 different ratios, while the character pair "type of colony center" is found to be segregated in 5 different ratios. Such segregation ratios suggest that color is the expression of 2 additive linked pairs of Mendelian factors and that type of colony center is the expression of a number of Mendelian factors. The evidence so far obtained is insufficient to show that the relative proportion of these segregation ratios is altered by a change in the external medium, but it is possible that the linkage between the two pairs of color factors is increased by raising the nitrogen-source concentration in the medium. By an extended investigation of the effects of the external medium chiefly on the segregation of 1 character pair, it has been possible to show that the onset and duration—as measured in nuclear divisions—of the process of meiosis is in part controlled by the external environment of the cell.

Pathogenic and cultural comparisons of strains of Rhizoctonia solani. O. H. Elmer.

Pure-line vegetative strains of *Rhizoctonia solani* were compared as to pathogenicity and cultural characteristics. Successive vegetative generations retained the cultural and pathogenic characteristics of their parent cultures. Certain strains differ so widely from others that they can be placed in distinct groups. The group most

frequently encountered produces necrotic lesions on potato stems. Individual strains exhibit distinct differences in cultural characteristics and in virulence. One strain rarely produces potato stem lesions but invests the stem with an abundant, dark mycelial felt. Another strain produces complete necrosis of infected potato sprouts, the infection frequently extending beneath the eye and causing seed-piece decay. Strains of a second group produce superficial, fleck-like lesions on potato stems and cause arrested apical growth of emerging sprouts even in the absence of lesions near the sprout tip. Such plants produce side sprouts whose apical growth may, in turn, be arrested. No evidence has been obtained that the arrest of growth is due to by-products from the parasite. On laboratory media these strains differ considerably from those of the first-mentioned group, producing numerous, small, white, mealy-appearing mycelial aggregations. Other strains occur that do not fall into the above 2 groups.

Growth of Phymatotrichum omnivorum in plant juices as correlated with resistance of plants to root rot. Walter N. Ezekiel, J. J. Taubenhaus, and J. F. Fudge.

Phymatotrichum omnivorum has been grown in series of cultures prepared with juices expressed from 4 monocotyledonous plants (corn, onions, cannas, and nut grass), resistant to root rot, and from 4 dicotyledonous plants (cotton, carrots, turnips, and sweet potatoes), susceptible to the disease. The oven-dry weight of the fungus mycelium produced in the cultures was determined. With autoclaved, nondiluted juices, growth of the fungus was markedly inhibited in juices from all the resistant plants, while profuse and heavy growth was obtained with juices from 3 of the 4 susceptible plants. However, with diluted juices from the resistant plants good growth was obtained. Monocotyledonous plants resistant to Phymatotrichum root rot apparently contain materials that, in sufficiently high concentration, can inhibit growth of the root-rot fungus, and the resistance of these plants to the disease probably is based, at least in part, on the presence of such materials.

Concentration of salts and soil reaction as affecting growth of the root-rot fungus in the soil. Walter N. Ezekiel, J. J. Taubenhaus, and J. F. Fudge.

Soil-culture studies in the laboratory were undertaken to determine whether the correlation of prevalence and destructiveness of *Phymatotrichum omnivorum* root rot with the soil reaction might be based on the amounts of calcium salts in soils, rather than the pH. In soils in which relatively small amounts of salts had been incorporated, growth of the fungus increased, in general, as the pH of the soil increased. Calcium-carbonate additions to acid soil caused greater increases in growth than the same weights of calcium or potassium added as nitrates, sulphates, or phosphates. This indicates that it was the increased alkalinity rather than additional calcium, potassium, phosphate, or nitrate ions that favored growth of the fungus. The results demonstrated also an inhibitory effect of high concentrations of soluble salts, such as potassium nitrate and phosphate. Toxicity of salts depended on the percentage of the salt in the soil solution rather than in the total weight of soil. Large additions of relatively insoluble salts, such as calcium carbonate and phosphate, did not inhibit growth. Specifically toxic materials, such as potassium carbonate and various disinfectants, inhibited growth even in lower concentrations.

The dissemination of cereal rust spores in the greenhouse by terrestrial invertebrates.

A. A. Granovsky and M. N. Levine.

The imported garden slug, Agriolimax agrestis, and spring tails, Collembola sp., have been observed to frequently visit and feed upon uredinia of Puccinia graminis.

Plant lice, thrips, fungous gnats, and other insects in feeding also come in intimate contact with the pustules of rusted plants and are found to carry rust spores on their bodies, thus serving as vectors. The rôle of various invertebrates in dissemination of urediniospores of cereal rusts in the greenhouse was determined experimentally. Healthy wheat plants were repeatedly infested with slugs that had previously been allowed to feed on rust pustules. Infections invariably occurred along the slug's trail, marked by mucous secretion. Urediniospores adhere to the secretion on the body of the slug and are thus disseminated from diseased to healthy plants. Infection has been obtained with two physiologic forms of wheat rust by using the slug's intestinal content and excreta as inoculum. The spores that passed through the slug did not lose viability and readily caused infection, although the incubation period was somewhat prolonged. Infection also was obtained by using the common greenhouse thrips, Heliothrips femoralis, which were previously allowed to come in contact with urediniospores in Petri dishes or on rusted plants.

(Cooperative investigations between the Division of Entomology and Economic Zoology, the Division of Plant Pathology and Botany, University of Minnesota, and the Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture.)

The sooty-blotch and flyspeck of the apple. A. B. Groves.

A considerable number of isolations of the sooty-blotch fungus have been made from apple-fruit material since the inception of the present studies 2 years ago. Isolation attempts with the flyspeck fungus have been more limited. Many difficulties have been encountered in the development of an isolation technique that would permit of a high percentage of success. However, a technique has been developed that has given successful results in over 50 per cent of the trials.

Numerous sooty-blotch-thallus types have been observed during these studies, some of which are quite distinct. The types observed varied from those possessing a fine and evenly growing thallus, producing few or no pleetenchyma, to types producing very conspicuous plectenchyma with inconspicuous interconnecting mycelium; from those having a finely reticulate thallus to some exhibiting strongly radial growth characters. Types have also been observed that penetrate and make some growth beneath the cuticle. Differences in cultural behavior have been observed in many of the various sooty-blotch isolations. These studies are not complete, however, and possible correlations between the several cultural types and the thallus types, as observed on the apple, await further investigations.

The association of bunt of wheat with loose smut and ergot. W. F. HANNA.

Bunt (Tilletia levis or T. tritici) and loose smut have been found in the field growing side by side in the same head of wheat. Usually the topmost spikelets contain a few bunt balls, whereas the lower ones are completely destroyed by loose smut. The mortality is high among seedlings inoculated with both T. tritici and Ustilago tritici. In greenhouse experiments with Kota only 44 per cent of the seeds inoculated with these 2 smuts produced mature plants, as compared with 97 per cent for uninoculated seeds and 74 and 72 per cent for seeds inoculated with U. tritici and T. tritici, respectively. Of the plants grown from seedlings inoculated with both T. tritici and U. tritici, 64 per cent produced heads infected only with U. tritici, 23 per cent heads infected only with T. tritici, and 7 per cent heads infected with the 2 smuts. Young bunted kernels of wheat may become infected with ergot. The upper part of the kernel is converted into bunt spores and the remainder into an ergot body. Kernels of Little Club and

Mindum having an ergot body and a bunt ball fused together in this way have been found.

Physiologic forms of loose smut of wheat. W. F. HANNA and W. POPP.

Two distinct physiologic forms of *Ustilago tritici* have been found in Manitoba. One form, collected on Reward wheat, infects heavily many of the common wheats but failed to infect the durum varieties Mindum and Pentad. The other form was collected on Mindum. The durums are susceptible to this form, whereas the common wheats tested are moderately resistant to it.

An analysis of variation in Botrytis cinerea by single-spore cultures. H. N. Hansen and Ralph E. Smith.

Forty-seven strains of fungi of the Botrytis cinerea type were isolated from various vegetation. From each of these strains 2 monospore cultures were made. From each of 8 of these, 25 single-spore cultures were made. In most of these strains the growth in all the tubes appeared uniform but in a few considerable variation appeared in regard to presence or absence of sclerotia, production of conidia, color and type of mycelium, and other features of gross morphology. There was much variation between the different strains. In 1 (marked X) 24 (96%) of the cultures (x) appeared uniform and like the parent, while 1 (a) (4%) varied decidedly in absence of sclerotia and type of mycelium. This strain was studied further in repeated single-spore isolations of successive generations. In each instance 25 monospore tube cultures on potato-dextrose agar were made and new cultures started at 10-day intervals. The test was continued to the F_4 generation. x continued uniform throughout. In the F_1 generation a separated into 7 (28%) like the parent and 18 (76%) of an entirely new type (b). b continued uniform to the F₄ generation. a separated in the F₂ into 12 (48%) of a, 7 (28%) of b, and 6 (24%) of a new type (c). From this point b continued uniform. In the F_3 generation a separated into 2 (8%) of x, 15 (60%) of a, 5 (20%) of c, and 3 (12%) of a new type d. c separated into 1 (4%) of x, 14 (56%) of a, and 10 (40%) of c. Like conditions have been found in the case of species of Phoma, Fusarium, and Ramularia.

Studies of the properties and host reaction of the onion to the yellow-dwarf virus. W. J. HENDERSON.

Yellow-dwarf virus, in sterile distilled water and stored at 29° C., is inactivated after 112 hours, and, in onion leaves stored at same temperature, after 100 hours. Thermal death point of the virus, when heated 10 minutes, lies between 75° and 80° C. Freezing at -10° C. for 10 minutes produced no effect on infectivity of the virus.

Healthy bulbs inoculated in the growing tips with 2 hypodermic injections of 0.75 cc. each, became 80 per cent infected. Three injections of 0.42 cc. each infected 72 per cent and 1 injection of 2 cc. infected 25 per cent of the plants.

Healthy onions, inoculated in leaves when 1½ inches high, expressed symptoms on 35 to 40 per cent of the plants during the current growth, and 50 to 60 per cent had masked expressions in next growth period. Plants 4 to 6 inches in height, when inoculated, expressed from 5 to 20 per cent disease during current growth and 25 to 40 per cent in second growth period. Plants 7 to 8 inches in height, when inoculated, failed to express disease symptoms in the first growth period but expressed 25 to 30 per cent during second.

Further studies on ripe rot of pepper. B. B. Higgins.

The causal fungus has been compared to specimens and cultures of Vermicularia capsici from India and the two have been found to be identical. Pimiento fruits inoculated with a culture of V. capsici received from India developed symptoms identical with the ripe-rot symptoms previously observed here. Cultures of morphologically-similar fungi from a number of other host plants have all proved either nonparasitic or weak wound parasites on Pimiento pepper. The fungus lives over winter on decayed peppers left in the field, also on the seed from such decayed fruits. It may persist in or on the surface of seed in storage at least 18 months. Histological study of inoculated pepper fruits has shown that the fungus spores produce appressoria on the end of short germ tubes. From these appressoria a thin penetration hypha passes into the host tissues usually within 2 to 4 days, under moist conditions. By this time the epidermal cells near the appressoria are killed. Up to this stage the development in green and ripe fruits appears similar, but in green fruits there is little further development of the fungus. The mycelium develops very slowly in these dead cells until the fruit begins to ripen. The fungus then begins rapid growth and a decayed spot is soon evident. In inoculated ripe fruits spots develop in 6 to 10 days.

Studies on Bordeaux deposition. George L. Hockenyos and George R. Irwin.

There is described a laboratory technique of applying a definite amount of spray to selected leaves. This uses the principle of a pendulum shutter cutting off a constant jet of spray. Methods of sampling and analyzing are described whereby both the amount deposited per unit area and the uniformity of deposit are determined. It is shown that the addition of a wetting agent increases the uniformity of deposit but does not decrease the amount deposited per unit area. The amount of deposit is shown to correspond closely to that found by Guba. Dried blood albumin was used in most of the work but comparisons were made with soap and several wetting agents.

The relation of maturity of seed to seedling-blight susceptibility in dent corn. P. E. HOPPE, J. R. HOLBERT, and J. G. DICKSON.

The expression of resistance to seedling blight in corn caused by Gibberella saubinetii is affected by many environmental factors. It has been found that the environment during the growth and maturation of the mother plants greatly affects the disease reaction of seedlings of the subsequent crop, when inoculated with G. saubinetti, regardless of the genetic potentiality for resistance in the strains. Results from experiments have shown that different degrees of maturity of the seed influence the disease reaction and the yield of the crop. Several hundred self-pollinations in inbred lines were made, the ears were harvested periodically, and the resulting seed was inoculated, planted, and rated for resistance to seedling blight. Results indicated that resistance to seedling blight increased with the maturity of the parent seed. However, the rates of increase in resistance varied at the different stages of maturation of the parent seed. Assuming the disease reaction of the seedling to be an index to the actual degree of maturity of the parent seed, it appeared that some strains, relatively slow in development in the earlier stages, ripened more rapidly and actually matured earlier than others that had previously appeared more mature. (Cooperative investigations between the Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, Wisconsin Agricultural Experiment Station, and Funk Bros. Seed Company.)

Avocado diseases in California. Wm. T. Horne.

Avocado production and marketing appear to be established on a permanent basis in California. The plant diseases that have influenced this development up to the

present time have been of a somewhat unusual and special character. Water injury or Melanorhiza causes death of trees. Tipburn is a symptom, usually associated with some phase of the alkali problem. Chlorosis and little leaf apparently parallel similar diseases of citrus. Sun blotch is an infectious chlorosis. An important canker of green stems has not yet been solved. Carapace spot of fruit is due to very early and slight surface injury. Thompson spot is a necrosis on the lower end of green fruit, followed by internal changes, which cause loss of the affected fruits; cause not determined. End spot is a breakdown of mature or overmature fruit. Spoilage of mature fruit on the tree or of fruit that has been removed and is in process of softening is caused, in approximately the given order with regard to ripeness of fruit by Dothiorella, Colletotrichum, Fusarium, Alternaria, miscellaneous organisms, Rhizopus, and putrefactive bacteria.

Effects of root rot on the physiology of peas. James G. Horsfall, Z. I. Kertesz, and E. L. Green.

A study was made of the effect of pea root rot (a disease complex caused by a series of fungi) on quality, maturation, and chemical composition of canning peas. Peas from normal and diseased plants were compared by following, during the ripening period, the changes in size distribution, load necessary to crush a single pea, and dry-matter, ash, nitrogen, and crude-fiber content. At first, diseased peas enlarged more rapidly than normal but soon lagged behind and never attained normal size. Since the crushing load for a single pea was higher, size for size, on the same harvest date, for diseased than for normal peas, the quality of the diseased peas was lower. On an equal dry-matter basis, however, crushing load was lower for diseased than for normal peas. Possible reasons: (1) diseased peas dried out before they were filled, thus being smaller than normal; (2) the construction or composition of the dry-matter stuff was weaker; (3) ash and nitrogen contents were always lower. Crude-fiber content, however, seemed to bear no significant relation to healthiness. Growth curtailment and lowered quality in diseased peas seemed to be due to lowered water content and consequent decreased metabolism and translocation.

The fungi present on the surfaces of normal apples and their relation to decay. GLENN A. HUBER.

The surfaces of normal apples obtained from 4 important apple-growing districts of Washington—Kennewick and the Spokane, Yakima, and Wenatchee valleys—were studied to determine the fungi present. One hundred and twenty-four species, or forms, belonging to 29 genera, not including 23 unclassified, non-rot-producing forms, were isolated from the surface of normal apples. Fifty-eight of these species, or forms, were found capable of causing distinct decay when inoculated into sound, ripe Jonathan apples, of which 34 were capable of causing decay when incubated at 0-2° C. Over 20 species belonging to 14 genera were obtained that had not been previously recorded on apples in the State of Washington. The following fungi, found capable of causing decay of apples, are reported for the first time: Sporormia sp., Pyrenochaeta sp., Chaetomella sp., Aspergillus sp., verticillium sp. (Forms 1, 2, and 3), and Podosporiella spp. (Nos. 1 and 2).

Stewart's disease of corn. S. S. IVANOFF.

Studies on the Stewart's disease of corn, caused by Aplanobacter stewarti, have shown that the pathogen may enter the roots of corn plants grown in artificially infested soil through wounds produced artificially or through wounds produced by white grubs, the larval stage of Phyllophaga sp. The location of the pathogen in the corn tissues

was determined by means of histological and isolation studies. In the leaf tissue the bacteria escaped from the vessels and entered the parenchyma. Their activities within ruptured cells and between cells caused discoloration, plasmolysis, and death. In the kernel the bacteria were observed in vessels and in adjoining cavities of the chalazal region, between the testa and the aleurone layer and between the endosperm cells. In the tassel the bacteria were located in the vessels of rachises, rachillas, glumes, and filaments. The bacteria were isolated from exudate on leaves and the water at the base of unfolding leaves, from the glumes, anthers, and pollen of diseased plants, and from corn stubble overwintered in the field. A selective medium was developed with which the bacteria were recovered from the soil.

Scab and canker found on Citrus in herbaria of England and the United States. Anna E. Jenkins and H. S. Fawcett.

Citrus material has been examined at the Herbarium, Royal Botanic Gardens, Kew, and the Department of Botany, British Museum, South Kensington, London, England, and Gray Herbarium and the Arnold Arboretum, Harvard University, Cambridge, Mass., the New York Botanical Garden, New York, N. Y., the Philadelphia Academy of Natural Sciences, Philadelphia, Pa., and the United States National Herbarium, Washington, D. C.

The examinations revealed the occurrence of both Citrus scab and canker at dates and in localities not previously recorded. Scab was found on collections from Japan made in 1862, from India in 1868, and from China and Korea at later dates. Also, what is apparently scab was present on a specimen from Java collected as early as 1840. Canker was found on collections of Citrus recorded from Java as early as 1842 to 1844, from the Philippines in 1903, and from other countries at more recent dates.

The presence of starch near wounds in Burley tobacco plants.—Burt Johnson.

In the preparation of concentrated suspensions of the virus of tobacco mosaic it was necessary to measure the strength of the various materials in the most quantitative manner possible. Leaves were punctured with virus-bearing pins, according to a method described by Holmes, and then by the iodine reaction, the amounts of virus present were determined by the percentages of wounds having excessive amounts of starch around them. When Burley tobacco was used as the host plant there was no constant diminution in the number of local lesions with increase in dilution of the virus. Juice from healthy plants used as inoculum gave essentially the same results as juice from diseased plants. Different chemical treatments of the virus did not appreciably affect the percentages of lesions found. Water inoculated into the plants or wounds of several kinds gave numbers of lesions about equal to those found when virus was inoculated into the plants. If the leaves had little or no starch in them to begin with, lesions, regardless of the inoculum used, were few in numbers and very indistinct. Other plants were tested in a similar manner and like results were obtained according to whether or not the leaf was well supplied with starch.

Verticillium wilt of chrysanthemums. LEON K. JONES and GLENN A. HUBER.

Verticillium wilt is prevalent in greenhouses in Washington State and 100 per cent damage to the crop has been noted with certain varieties. The following varieties have shown marked susceptibility to the disease: Adrian's Pride; Chadwick; Distinction; Dr. Enguehard; Favorite; Gladys Pearson; Golden Measure; Helen Frick; Justrite; Mrs. Simpson; October Rose; Mistletoe; Quaker Maid; Rose Glory; Rose Royal; Seidewitz; Silver Sheen; Whittier; and Wolf's Pink. The following varieties appear to be highly resistant to the disease: December Beauty; December Glory; Early Frost; Friendly

Rival; Gold Lode; Golden Queen; Honey Dew; Improved Golden Glow; Indianola; Lustre: Monument; Wm. Turner; W. H. Waite.

Preliminary investigations tend to show that the organism is carried in the cuttings taken from diseased plants of the more susceptible varieties. Control recommendations should emphasize the use of cuttings from healthy plants as well as soil sterilization.

Bactericides in relation to Bacillus amylovorus and fire-blight control. G. W. Keitt, Luther Shaw, and A. J. Riker.

Relative toxicity of various chemical compounds and spray preparations to Bacillus amylovorus was studied, in which a modified Rideal-Walker technique was used. Silver and mercury salts showed outstanding toxicity; copper and cadmium salts, a low intermediate degree; nickel and iron salts, relatively low toxicity. Compounds of mercury varied much in relative toxicity, certain organic compounds and mercuric chloride being most toxic. Bordeaux mixture, zinc sulphate-lime mixture, and lime, respectively, killed the bacteria at concentrations lower than commonly used in sprays.

Spray programs were applied to large apple trees in which suitably located blossom clusters had been inoculated by a standardized procedure to furnish an abundant natural fire-blight inoculum. Abundant blossom blight developed on nonsprayed inoculated trees, the chief natural infection period coming at late full bloom, when there was a 2-day-rain period. The spray application made at full bloom seemed to be the most effective. Bordeaux mixtures, 3-6-50 and 1-3-50, excellently controlled blossom blight. However, the stronger Bordeaux severely russeted the fruit. In a single trial lime, 3-50, gave very promising results. Various mercurial sprays were less effective, reducing blossom blight by approximately ½ to $\frac{2}{3}$. Recommendations are withheld pending further work.

Fasciation in red pine. RAYMOND KIENHOLZ.

A red pine, *Pinus resinosa*, about 20 feet high and 18 years old, growing in a plantation of the Yale Forest at Keene, N. H., was found to exhibit extensive fasciation of the leader and upper laterals during the past 4 years of its growth. Because of the rarity of fasciation in conifers in general and pine in particular, it is of interest. The upper part of the tree is bushy, the fasciated branches being bunched and bearing great masses of needles. The leader seems particularly subject to fasciation and, when fasciated, a lateral bends upright to take its place, only to become fasciated in turn.

Moisture of shelled corn in relation to fungus growth. Benjamin Koehler.

Shelled yellow-dent corn was placed over calcium chloride solutions of different concentrations in closed vessels, some series aerated, others not. Twelve different humidities were provided in which the corn moistures ranged from 9.6 to 32.0 per cent (wet basis). In some series internally infected corn was surface sterilized and maintained free from contamination so as to observe better the effect of certain infections. All series were kept for 2 months at room temperature before final observations were made. No fungus growth occurred at 14 or less per cent moisture in these experiments. Aspergillus growth was found at 14.9 and above. "Blue eye," a Penicillium growth over the germ but beneath the pericarp, occurred frequently at 19 to 21 per cent. Diplodia zeae became active at 21.2 and Fusarium moniliforme, Gibberella saubinetii, and Basisporium gallarum at 22 to 23 per cent. Diplodia grew more extensively and was more destructive at marginal and higher moistures than the 3 last-named fungi. Aeration had little effect on determining the minimum moisture range of the fungi observed, but in some cases lack of aeration checked their growth.

Local lesions in Aucuba mosaic of tomato. L. O. Kunkel.

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Studies on Aucuba or yellow mosaic of tomato obtained from England show that the virus of this disease produces necrotic local lesions in leaves of the following species and varieties of Nicotiana: N. acuminata, N. alata, N. glutinosa, N. langsdorffii, N. rusbyi, N. rustica, N. suaveolens, N. sylvestris, N. tabacum var. Adcock, N. tabacum var. Burley, and N. tabacum var. Little Oronoca. It produces chlorotic local lesions in the following species and varieties: N. paniculata, N. tabacum var. Connecticut Seed Leaf, N. tabacum var. Macrophylla, and N. tabacum var. Purpurea. It also produces chlorotic local lesions in leaves of the following varieties of tomato: Beef Steak, Early Freedom, Globe, Golden Queen, John Baer, June Pink, Matchless, Red Pear, Red Plum, Red River, Stirling Castle, and Yellow Cherry. The production of local lesions is associated with multiplication of the virus at the point of inoculation. Aucuba mosaic differs from the ordinary tobacco mosaic in its capacity to produce local lesions in the tomato and in certain species and varieties of Nicotiana.

Factors affecting infection and decay of sweet potatoes. J. I. LAURITZEN.

Host response of Citrullus vulgaris to Colletotrichum lagenarium. Duke V. Layton.

Colletotrichum lagenarium produced maximum infection on Citrullus vulgaris in the greenhouse at 20 to 28° C. and a relative humidity of 93 per cent. Less infection was secured at 17° and none at 15° C. with nearly the same humidity (90 per cent).

The reaction to the anthracnose organism of 43 varieties from 9 countries, 416 mass selections from the varieties Kleckley Sweet, Conqueror, and Halbert Honey, and 46 hybrids was determined by indexing in the greenhouse. Of these, 116 were given further trial in the field. The citrons (particularly Majorta, an African forage melon) showed a high degree of resistance, but most of the oriental varieties were markedly susceptible. The F_2 and F_3 generations of 5 citron-watermelon hybrids were appreciably less susceptible than commercial watermelons. Several inbred segregates from chance hybrids of Conqueror have shown marked resistance to Colletotrichum lagenarium. These are selections from Iowa Belle (Q21), Iowa King (Q23), and Q20, which are also edible and wilt resistant.

New parasites of cereal rusts. M. N. LEVINE, A. A. GRANOVSKY, and J. G. LEACH.

In the course of experiments dealing with the dissemination of rust spores by the garden slug, Agriolimax agrestis, wheat seedlings in the greenhouse were inoculated with urediniospores of Puccinia graminis tritici that had passed through the intestinal tract of slugs. Neither the virulence nor the specialization of the physiologic forms tested was changed. However, many of the resulting pustules were parasitized by a fungus and a bacterium. The effect was most pronounced on rusted seedlings, but the rust on adult plants also was severely affected. The organisms were isolated in pure culture and the action of each has been studied separately. Neither the bacterium nor the fungus appears to be pathogenic to healthy plants. High relative humidity is a prerequisite for the growth of the fungus parasites; whereas the bacterium thrives well under normal climatic conditions, whether in the field or in the greenhouse. All of the cereal rusts are apparently susceptible. The identity of the parasites has not yet been determined.

(Cooperative investigations between the Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, and the Division of Entomology and Economic Zoology and the Division of Plant Pathology and Botany, University of Minnesota.)

Present status of the Dutch elm disease. O. N. Liming.

In the summer of 1931 4 trees affected with the Dutch elm disease were found in Cleveland, Ohio, thus making a total of 8 cases in America. The original source of the disease in this country has not been found. About 600 diseased elm trees were examined. Several other elm diseases were encountered in the investigation. In inoculation experiments, infection of American elms was secured by several hypodermic injections at one level and by incision wounds. Chinese and Siberian elms, inoculated with Graphium ulmi, showed only internal symptoms of the disease.

Graphium ulmi survived the winter in artificially inoculated twigs placed outdoors under a bell jar. In a branch in which only the 1929 ring was discolored, there was no evidence of the fungus passing outward to the next year's ring. The fungus in a diseased tree trunk, placed on moist soil in the greenhouse, produced coremia on the bark and in borer tunnels. Branches from a diseased tree became sterile after 2 months in a dry laboratory. The fungus has its highest growth rate between pH 5.0 and 6.0 and at 22° C.

(Cooperative investigation between Division of Forest Pathology, Bureau of Plant Industry, and Ohio Agricultural Experiment Station.)

Progress in the control of stain and mold fungi that discolor stored lumber and logs. R. M. LINDGREN, T. C. SCHEFFER, and A. D. CHAPMAN.

Sapwood of both logs and lumber is liable to stains (caused by species of Ceratostomella and imperfect fungi with dark hyphae) and superficial molds (mainly Moniliaceae). These have been a considerable commercial handicap in the South, even where the best handling methods were employed. Following extensive small-scale elimination trials with fungicides, commercial tests were conducted in 1930 and 1931 at mills in several States. In most places satisfactory control has been secured on hardwoodsgum (Liquidambar), poplar (Liriodendron) and oak—with 5 per cent borax solution and on both pine and hardwoods with 0.01 per cent ethyl mercuric chloride; good results have also been secured with sodium salts of tetrachlorphenol or orthophenylphenol, or mixtures of the two, in concentrations of approximately 0.4 to 0.5 per cent. At the larger mills lumber is carried by the regular conveyors through a vat containing the cold solution; costs of the fungicides approximate 12 cents per M board feet, or only about 3/5 cent per hundred square feet of surface protected. Many mills have now adopted one of the new fungicidal treatments. In small-scale tests 0.4 per cent sodium 2-chlororthophenylphenolate appeared promising on pine and 3.6 per cent boric acid was effective on hardwoods.

Some of the treatments have been found effective in small-scale tests as a spray in preventing the deterioration caused by stain and decay fungi in logs stored during the winter months.

Etiological concepts of plant pathology. George K. K. Link.

Engrossed in control problems, dominated by germists, and neglectful of theoretical development of its etiological subject matter, phytopathology is considered "applied mycology" and has failed to gain recognition as a botanical science commensurate with that afforded physiology and genetics. As a pure science it is not motivated by practical considerations, includes all pathic events, and has more in common with physiology and genetics than with mycology.

Adequate development of etiological pathology involves recognition that the organization of the pathic organism plays a pathogenic rôle in practically every pathic event. Further analysis of the antecedents of a pathic event occurring at a given instant and

place involves consideration of (1) the genetic constitution of the place; (2) the past history of the place; (3) the correlative influences acting on the place; and (4) the immediate influence of the external environment (including pathogenic organisms). It involves use of the concepts and data of genetics, developmental physiology, and biochemistry as much as those of ecology (host-parasite relations and relations of nonliving environment factors to pathic and pathogeneic organisms).

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This approach does not ignore "hereditary" and noninfectious diseases and integrates susceptibility, infectivity, and virulence phenomena in a biologically-sound etiological system.

The precipitin-ring test applied to fungi. George K. K. Link, Adeline DeS. Link, George L. Cross, and Hazel W. Wilcox.

A procedure has been devised that permits application of the precipitin-ring test in the study of proteins of fungi. Fungus mats grown in protein-free synthetic medium, upon proper washing, drying, grinding, and extraction with fat solvents, yield highly antigenic extracts in 0.85 per cent NaCl solution. The nitrogen content of these extracts has been determined by micro-Kjeldahl digestion and their protein content calculated. The extracts remain clear, do not cloud, nor give nonspecific precipitates with normal serums, but give definite and graded precipitin rings when progressively higher dilutions in 0.85 per cent NaCl are layered on homologous and heterologous antiserums.

Details of procedure have been determined for each step in production of antiserums (preparation and dosage of antigens, interval, frequency, and method of injection into rabbits), and in execution of tests (relative merits of various mediums of dilution and of types of layering; rôle of pH of antiserums and test antigens and of time and temperature factors).

The method has been applied to 23 species and varieties of Fusarium and to 1 species, each, of Cylindrocarpon, Gibberella, Hypomyces, Neurospora, Ramularia, and Sclerotinia. The precipitin-ring test gives promise of applicability in mycological and mycophytopathological studies.

(Aided in part, since 1929, by a grant to the University of Chicago from the Rockefeller Foundation.)

Parasitism of Diplodia zeae on the crown of the corn plant. George L. McNew.

On land previously cropped to corn, plants from nearly disease-free seed had 12 per cent of crown infection with Diplodia zeae Lev., and plants from treated Diplodia-infected seed had 18 per cent. One hundred per cent of the plants grown in steamed soil infested during the seedling stage with pure cultures of D. zeae became infected. Less infection resulted and progress in the crown was retarded when the soil was infested later in the growing season. At maturity 80 per cent of the plants showing severe infection of the crown were invaded to the second internode. Under similar conditions, except that the inoculum was omitted, the same symptoms were produced on plants grown from seed naturally infected with D. zeae. A series of plants showing 81 per cent mesocotyl infection, but escaping seedling blight, showed 80 per cent crown infection when the crop was mature. The pathogen invades the crown either from the soil or from infected seed.

An experiment on the influence of soil moisture on crown infection indicated that root reduction was greatest in compost soil at 45 per cent of the water-holding capacity (42 per cent). In a second experiment 80 per cent infection developed at 45 per cent soil moisture, which was higher than occurred at either 30 or 85 per cent soil moistures.

Cyclamen stunt. L. M. MASSEY and PAUL E. TILFORD.

This disease was observed in New York in 1926 and in Ohio in 1929 and is known to occur in Pennsylvania, New Jersey, and California. Affected plants are conspicuously stunted but not quickly killed. Leaves are small and may be yellow. The petioles and peduncles are shorter than normal and the flowers characteristically open below the leaves. Necrotic areas, reddish brown in color, are found in the tissues of the corm. They are typically confined to the crown of the corm but may extend throughout—even for short distances into the petioles, peduncles, and the larger roots.

Cyclamen stunt is caused by an undescribed species of Cladosporium, for which the name C. cyclaminis n. sp. is proposed. Successful infection experiments have been conducted both with spores and mycelium. The fungus grows slowly in culture, producing on potato-dextrose agar a raised or stroma-like greenish black thallus commonly covered with a grayish white aerial mycelium. Spores are formed in culture in from 4 to 7 days, aerogenously, singly or in short chains, on short rarely-branched conidiophores. They are hyaline, becoming somewhat brown with age, typically 0- to 1-septate, and average $17.75 \times 4.3 \mu$.

Control of basal rot of narcissus. L. E. MILES.

In 1928, 1929, and 1930 experiments were conducted on control of a basal rot of narcissus bulbs caused by a species of Fusarium. Preliminary tests showed conclusively that the disease is disseminated by the hot-water treatment as required by the Federal Quarantine Act against the bulb eelworm and bulb flies. Mercuric chloride, formaldehyde, Semesan, and Ceresan used in conjunction with the hot-water bath prevented this dissemination and gave varying degrees of control of the rot. Ceresan, at a concentration of 1: 200, gave 99 per cent healthy bulbs; formaldehyde, concentration 1: 240, 88 per cent healthy; with mercuric chloride and Semesan intermediate as compared with 82 per cent healthy in the dry, untreated checks and 59 per cent healthy in the bulbs undergoing the unmodified, hot-water bath.

In treatment of bulbs immediately after or without hot-water treatment Calogren 1: 20, used as a dip, gave much better control of disease than any other chemical tested. All treatments yielded large increases in yield over the untreated bulbs. This increase was directly proportional to the degree of disease control attained. Dust treatments proved less efficient than did the liquid dip and soak treatments.

Verticillium wilt of cotton in Mississippi. L. E. MILES and T. D. PERSONS.

In the fall of 1929 a wilt or hadromycosis of cotton, caused by a species of Verticillium, tentatively determined as V. albo-atrum was discovered on experimental plots at the Delta Branch Experiment Station at Stoneville, Miss. In the fall of 1930 and the present year a survey was made by means of actual examination of fields and by soliciting specimens from county agents, State Plant Board inspectors, and others to secure an idea of the comparative distribution and prevalence of the trouble as compared to Fusarium wilt, from which it is, with our present knowledge, indistinguishable except by cultural methods.

All specimens collected or received were cultured and the presence or absence of Fusarium vasinfectum or Verticillium recorded. The specimens cultured represent collections from 30 of the 82 counties in the State, widely scattered on all types of soil. One-sixth of the collections have yielded the Verticillium fungus. One-fifth of those showing Verticillium have given cultures of Fusarium, also. All others have shown only Fusarium. Verticillium wilt has been found in 8 counties, namely, Tallahatchie, Bolivar, Sunflower, Coahoma, De Soto, Washington, Tunica, and Leake. All, with the exception of Leake, are in the Delta section.

The genetics of Ustilago zeae. M. B. MOORE.

A study was made of the gametic (F_1) segregates from crosses between 1 monosporidial line and each of 2 others that had contrasting cultural characters. All of the F_1 lines isolated from the 2 crosses fell into 17 cultural groups, 49 from 1 cross falling into 12 and 38 from the other into 13 groups. A few F_1 lines were almost identical with one or another of the parental lines. All characters studied, including sex, apparently were governed by multiple factors. Segregation of factors for cultural characters occurred in the second division of the fusion nucleus or began in the first and was completed in the second. Occasionally segregation may have occurred in still later divisions. Three different combinations of the F_1 lines from a single chlamydospore differed greatly in their ability to form chlamydospores on corn plants, even though all produced abundant galls consisting of fungus mycelium and hypertrophied host cells. The number of spores formed varied with the variety or line of corn inoculated. One combination, although producing numerous large galls, formed chlamydospores very sparingly and only in occasional galls.

Sporulation of 5 species of Cercospora in pure culture. CLATUS M. NAGEL and S. M. DIETZ.

The failure of many species of Cercospora to sporulate abundantly in pure culture has been one of the limiting factors in studying the biology, classification, and cross inoculation of the species of this genus. During the past 9 months transfers from stock cultures of Cercospora beticola, C. dubia, C. davisii, and C. zebrina have been grown on 30 different media. During this time 40 successive transfers were searched for conidia as soon as they showed vigorous growth, and no conidia were found. During the summer of 1931 Cercospora was isolated at Kanawha, Iowa, from the following hosts: Beta vulgaris, Setaria glauca, Melilotus officinalis, Chenopodium album, and Physalis pubescens. Cultures of all 5 of these species on potato-glucose agar sporulated 48 hours after the original isolation. Ten successive transfers with conidia have been made from each of these cultures at 5-day intervals held at 24-27° C. In all of the cultures abundant conidial production occurred over the entire surface of the culture. Isolations made from B. vulgaris sporulated more abundantly on sugar-beet-leaf agar than on potato-glucose agar. Stock cultures of Cercospora beticola, C. dubia, C. davisii, and C. zebrina produced no conidia when exposed for different lengths of time to ultraviolet irradiation.

A greenhouse tomato hybrid somewhat resistant to Cladosporium leaf mold. A. G. Newhall.

Out of 4 English tomato varieties, procured from Dr. Bewley in 1928, two possess considerable resistance. Infection can occur but is delayed. The plants seem to be hypersensitive, so that very little sporulation takes place. Secondary infections are thus much delayed. Since none of the fruits of the English varieties Satisfaction and Main Crop seem to have the size and flesh characters demanded by the American markets, they have been crossed with Bonny Best and a pink Marglobe (Marhio). In the F₁ generation susceptibility was dominant. Seedlings of the F₂ generation were inoculated and the susceptible ones discarded. Some of the resistant ones were grown in ground beds until their 8th cluster had matured. Yields varied between 5 and 12 pounds per plant. Final resistance also varied somewhat as the plants aged.

Two commercial growers have been further testing the F₃, F₄, and F₅ generations. The aim has been to develop a midsize red tomato. Complete resistance has not been obtained, but a strain has been selected that is slower in developing the disease under

greenhouse conditions in New York State. Back crosses have been made to increase the size of fruit.

Root and storage rot of celery caused by Phoma apiicola. A. G. NEWHALL.

Late celery from certain muck lands in New York develops a soft, dark, greenish butt rot, after a few weeks in commercial cold storage (0° C.), rendering it unfit for market. This rot, caused by *Phoma apiicola*, is easily distinguished by its greenish color and less watery consistency from that caused by *Sclerotinia* sp. There is no evidence of disease at the time of harvesting the crop and placing it in cold storage in October. No important evidence of resistance to the storage rot has been found among 20 varieties tested, all showing from 18 to 90 per cent decay within 9 weeks.

By growing plants in artificially inoculated muck soil held at different temperatures (14°, 19°, 29° C. \pm 1½°) and different moistures (dry, medium, and wet) the root rot has been studied under a variety of conditions. Soil moisture had little influence. The fungus grows slowly and has a low optimum (18° C.) and a very low minimum temperature in pure culture. Isolations have been made from the roots of plants showing that there is very little evidence of root rot. On placing these plants in cold storage, however, at a high humidity, they decay almost completely in 7 weeks.

Variability of Venturia inaequalis in cultural characters and host relations. D. H. Palmiter.

Monoconidial cultures of Venturia inaequalis, isolated from 14 apple varieties from Wisconsin, Oregon, Michigan, and New York, showed cultural differences not correlated with the locality or variety from which they were isolated. Differences were of the following types: amount of aerial mycelium, margin of colony, rate of growth, and abundance of conidia or ascocarps produced in culture. In greenhouse inoculations infection occurred only on Malus species. Amelanchier, Aronia, Cotoneaster, Crataegus, and Sorbus species were not infected. Malus toringoides, M. sieboldii, M. ioensis, M. sargentii, M. niedzwetskyana, M. baccata, M. coronaria, M. tschonoskii, and M. robusta were each infected by one or more of the cultures. Malus floribunda, M. angustifolia, M. arnoldiana, and M. theifera were not infected. Under the conditions of the experiments Yellow Transparent was infected by 5 cultures and resistant to 4; McIntosh, infected by 2 cultures and resistant to 3; Dudley and Missouri Pippin, infected by 3 and resistant to 2; Hubbardston Nonsuch, infected only slightly by 3 and resistant to 2 of the cultures. The other 15 apple varieties inoculated were infected by all of the cultures.

Infection studies with Mycosphaerella fragariae and Diplocarpon earliana. A. G. Plakidas.

From a large number of tests it has been shown that infection on strawberry leaves with Mycosphaerella fragariae and Diplocarpon earliana takes place primarily, if not exclusively, through the epidermis on the lower side of the leaf. Inoculations were made by smearing spore suspensions on either the upper or lower leaf surfaces with a camel'shair brush. In the case of Mycosphaerella ingress is stomatal, and there appears to be a correlation between the amount of infection and the number of stomata on the leaf surfaces. With the variety Klondike the average number of stomata on the upper and lower leaf surfaces was found to be 3.16 and 147.77 per square millimeter, respectively. The mode of ingress in the case of Diplocarpon has not been determined definitely, but it does not appear to be stomatal. The fact that infection takes place primarily through the lower surface of the leaf may possibly be explained by the difference in the thickness of the cuticle and epidermal layers on the upper and lower surfaces, respectively.

The "June yellows" of strawberries. A. G. Plakidas.

The name "June yellows" is suggested for a disease or a peculiar yellow condition of the strawberry, which occurs in the Northeastern States and in Canada. This name is applicable because the symptoms are more pronounced in early summer. From certain of its symptoms, including the yellowing, mottling or marbling of foliage, stunted growth, and vegetative transmission from a mother plant to all its daughters through runners, the June yellows resembles diseases of the virus type. All attempts, however, to transmit it by juice inoculation and leaf mutilation, by the use of soil, by insects [white flies, leaf hoppers, and aphids—Aphis forbesi and Capitophorus fragaefolii (?)], and by bridge grafting have thus far failed. The bridge-grafting technique has been developed so that a complete union has been obtained with 75 per cent of the grafts. A certain percentage of plants growing from seeds from affected plants finally show the yellows symptoms, though these are not evident during the first year. The disease has been found in Massachusetts, Maryland, New York, New Jersey, Michigan, Ohio, Wisconsin, and Canada on one or more of the following varieties: Eaton, Beaver, Howard 17, Mastodon, Peerless, Van Dyke, Vineland seedling 2,532,192, Superb, and 23 different seedlings of the U.S. Department of Agriculture.

Reaction of tomatoes to mosaic. R. H. PORTER.

A study was made with 27 pure lines of tomatoes, each representing a variety or type. From 5 to 8 plants of each line were inoculated in 2 sets with tomato-mosaic virus. All of the plants in lines 11 and 13 developed symptoms in 10 and 11 days, respectively. In one line, 838-4, 2 plants out of 8 showed symptoms 11 days after inoculation and 4 more were infected the following day. The 2 remaining plants developed symptoms in 20 days. Five inoculated plants of line 398-4 developed no symptoms, but the virus was present, as was proved by transfers back to highly susceptible plants. Repeated trials with this line gave the same results. Fifty-nine plants of an F₂ generation (line 574-4), in which line 398-4 was one of the parents, were inoculated with tomatomosaic virus and 12 remained free of symptoms.

Plants of 20 pure lines were inoculated with the virus of yellow-tomato mosaic (probably identical with Johnson's tobacco virus 6) and all of the plants in 3 lines remained free of outward symptoms.

Some diseases of wild potatoes in Mexico. Donald Reddick.

Blight, caused by *Phytophthora infestans*, was found on *Solanum verrucosum* from the State of Morelos. Rust, caused by *Puccinia pittieriana*, occurs abundantly on *S. demissum* at El Desierto in the Federal District but was not encountered elsewhere in the *tierra fria*. Spot, a disease of unknown cause, is generally prevalent on several tuber-bearing species. Viroses and tuber diseases either were not encountered or else were not recognized on wild plants.

Basisporium dry rot of corn. Chas. S. Reddy.

Injury by Basisporium gallarum is associated with cessation of translocation within the corn plant. It occurs at the time of germination, after normal maturity, and when plants die prematurely from cold or other causes. Basisporium-infected seed dies within a few days after it is planted in soils slightly below the temperature range for germination. The organism kills the germ before active translocation sets in. Therefore, poor field stands are correlated with cold soil temperatures at time of planting. Seed treatments are most beneficial under conditions of greatest injury by the organisms, such, for example, as cold soil at time of germination.

Seed-corn strains that germinate readily at low temperatures (below 11° C.) are injured little by Basisporium gallarum. Experiments on artificial inoculation of corn ears with B. gallarum indicated that natural inoculum was widespread at the time and that the number of infected ears was not significantly increased by artificial wound inoculations. With or without artificial inoculation, susceptible ears became infected and the resistant ears did not. The resistant ears are those having high hydrogen-ion concentration in the cobs. Cob reaction is an inherited character and promises a means of more easily breeding strains of corn resistant to Basisporium ear rot.

Clitocybe mushroom root rot—a new disease of citrus trees. ARTHUR S. RHOADS.

Approximately 150 citrus trees in Florida, comprising grapefruit, oranges, and tangerines on rough lemon rootstock, have been found attacked by root rot caused by *Clitocybe tabescens*. This disease appears to be of long-standing occurrence in Florida citrus groves but has not been differentiated heretofore from foot rot.

Clitocybe mushroom root rot resembles that caused by the honey agaric or oak-root fungus (Armillaria mellea) in habit of growth, production of rhizomorphs, appearance of the mushroom-like fruiting bodies, and preponderance on land where oak trees have occurred.

The discovery of mushrooms at the bases of the trunks led to the location of many attacked trees in which the healthy appearance of the tops did not suggest the occurrence of root rot. In the large number of attacked trees in which the root systems were investigated a good proportion of the lateral roots, and usually the taproot also, was found to be invaded by Clitocybe and often well rotted before the tops showed any evidence of decline. In most of the cases oak, and occasionally other hardwood roots, were found, which were extensively invaded by the mycelium of this fungus, these often being in contact with and serving to transmit the disease to the citrus roots.

Clitocybe mushroom root rot—a new disease of bananas. Arthur S. Rhoads.

Mushroom root rot, caused by Clitocybe tabescens, has been found attacking and killing banana plants at two localities in Florida. At Artesia, where this disease was attacking a considerable number of trees and other woody plants on hammock land that supported a heavy growth of oak and other hardwood trees prior to clearing, a number of clumps, each, of Dwarf Cavendish, Hart's Choice or Lady Finger, and Orinoco or Horse bananas were found attacked. An additional record of the occurrence of this disease of bananas was afforded by a specimen received from Nocatee.

The bases of the attacked plants were involved in a watery, brownish discoloration, with sheets of mycelium and a luxuriant development of rhizomorphs between the leaf-stalk bases, which comprise the trunk, and within the septate tissues of the same. The rhizomes of the attacked plants were permeated by numerous mycelial sheets. The development of the mycelium through the rhizomes serves to transmit the disease to other plants in the clump and eventually the entire clump is killed.

Pure cultures of the fungus associated with the disease have been grown and carried to fruition in comparison with cultures of *Clitocybe tabescens* isolated from several other plants killed by this mushroom root-rot fungus.

Two forms of fire blight and a new related disease. H. R. ROSEN.

On Jonathan apple trees 2 different forms of blight have been distinguished with reference to different sources of inoculum. One form, early or principal epidemic of blossom blight, bears no discernible relationship to hold-over blight, and the second or later form, exclusive twig blight, is directly related to hibernated cankers and blighted

twigs present on the trees. The first may appear in different degrees of severity regardless of the presence or absence of blight the preceding year; the second is intimately associated with last year's blight.

Another bacterial disease of pomes, hitherto unreported, the symptoms of which simulate fire blight to a considerable degree, exists in America. It consists of a blossom blight, a fruit rot, and a leaf spot, but has not yet been found to involve a twig or limb blighting under natural conditions, though twig blight is readily induced with pure-culture inoculations. The pathogen is closely related to Bacillus amylovorus but shows numerous distinct cultural, physiological, and pathogenic properties. It belongs to the group of bacteria all of which have been noted in the literature as being akin to B. amylovorus, consisting of B. barkeri Berridge, B. nectarophilum Doidge, and Pseudomonas prunicola Wormald.

Physiologic strains of Fusarium niveum. Bailey Sleeth.

The failure of the Conqueror watermelon to prove resistant to watermelon wilt in the different melon-growing sections has suggested the possibility that there are strains of the wilt organism with various degrees of pathogenicity. Cultures of Fusarium niveum were secured from Texas, South Carolina, North Carolina, Iowa, and West Virginia. During 1930-31, greenhouse experiments have been in progress to determine the relative degree of pathogenicity of the various strains of the wilt fungus to certain varieties of watermelons. The ability of the fungus to produce wilt when tested against 6 varieties of watermelons is indicated by the percentage of plants wilted. When tested on 6 varieties, strain 3 produced an average of 90-95 per cent wilting; strains 8 and 6, 85-90 per cent; strains 4 and 1, 80-85 per cent; strain 2, 70-75 per cent; strain 7, 65-70 per cent; strain 5, 45-50 per cent; and strain 9, 20-25 per cent.

The amount of wilting produced by a given strain of Fusarium niveum is not always uniform when tested against different varieties of melons. Twenty-three strains have been isolated, which exhibit culture characteristics indicating the possibility of as many physiologic forms.

Diplodia stalk- and ear-rot studies of dent corn. A. L. SMITH and J. R. Holbert.

The reaction of numerous inbred and crossbred strains of yellow dent corn to infection by Diplodia zeae was determined following hypodermic injections of a spore suspension into internodes of stalks and shanks. Inoculations were made late in August. Extent of spread of infection, including ear rotting, was noted early in October. Significant correlations were found between extent of spread in stalks and extent of ear rot from shank inoculations. The coefficients of correlation between the extents of infection in cortical tissues and pith tissues following hypodermic inoculations and percentages of Diplodia-rotted ears at harvest in the same crossbred strains grown under field conditions and exposed to natural inoculation were +.84 ± .05 and +.73 ± .09, respectively. The results suggest that stalk inoculations with D. zeae may be useful in determining relative resistance of stalks and ears of inbred and crossbred strains to Diplodia. Stalk tissues of strains easily injured by exposure to artificial cold in field refrigeration chambers were more quickly invaded by Diplodia than were comparable tissues of strains possessing greater cold resistance. (Cooperative investigations between the Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, Wisconsin Agricultural Experiment Station, and Funk Bros. Seed Company.)

Seed transmission of Fusarium wilt of pea. W. C. SNYDER.

Seed transmission of pea wilt (Fusarium orthoceras) has been demonstrated in greenhouse trials. Untreated virgin soil from a source previously shown to be free of

the wilt organism was planted consecutively to 5 series of seed of which the first 4, totaling some 8,000, were harvested from wilt-infected plants and the 5th from healthy plants.

During the run of the 4th series, 4 plants in different parts of the seed bed wilted, and the organism was isolated. Additional wilting occurred in the 5th series, indicating the fungus had become established in the soil. The incidence of seed transmission is apparently rare. It seems clear, however, that the fungus is occasionally carried on or in the seed. This serves to explain some observed spread of the disease into widely separated areas.

Isolations of the causal organism from various parts of the United States have not revealed, so far, any differences in their pathogenicity upon susceptible varieties of pea nor in their lack of ability to cause the disease upon resistant varieties, even though considerable variation in cultural characters between isolations from within a given locality, as well as between those from different regions, has been obtained.

Distribution of physiologic forms of Puccinia graminis tritici in the United States and Mexico in relation to rust epidemiology. E. C. Stakman, Lee Hines, Harry G. Ukkelberg, and Wallace Butler.

From 1929 to 1931, inclusive, 27 physiologic forms of Puccinia graminis tritici have been identified from about 1,650 collections made in the United States and Mexico. Forms 11, 21, 36, 38, and 49 were by far the most prevalent (averaging almost 90 per cent of all collections) in each of the 3 years, although the order of prevalence of the individual forms varied with the year. Forms 49 and 38 were abundant in Mexico, the latter, which is only weakly pathogenic to the hard-red-spring wheats, constituting about 65 per cent of all collections. Forms 11, 21, and 36, on the other hand, were found very rarely in Mexico. All 5 forms were fairly prevalent in the spring in Texas, where some of them apparently overwintered in the uredinial stage. Results of spore-trap exposures and observations on the northward spread of rust indicate that urediniospores of these forms were carried from Texas northward by high winds. There is abundant evidence also that much inoculum came from barberries in the Northern States. (Cooperative investigations between the Divisions of Barberry Eradication and Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, and the Minnesota Agricultural Experiment Station.)

Physiologic forms of Puccinia graminis produced on barberries in nature. E. C. Stak-Man, Lee Hines, Ralph U. Cotter, and M. N. Levine.

Many new physiologic forms of *Puccinia graminis* have been produced experimentally by hybridization between existing forms, and many segregates from certain "selfed" heterozygous forms have been isolated from aecia. Presumably, hybridization on barberries also occurs in nature A survey, therefore, has been made of varieties and physiologic forms occurring on barberries and near-by gramineous hosts with primary infection. All North American races or varieties of *P. graminis*, except *P. graminis phleipratensis* develop readily on barberries. The following isolations have been made: 7 forms of *P. graminis secalis*; 17 of *P. graminis tritici*, of which 4 were previously unknown, although 1 was subsequently found on wheat; and 3 forms of *P. graminis avenae*, 1 of which, form 8, has been found only on oats near barberry. Evidently, new physiologic forms are being produced on barberries in nature, and, as mutation in pathogenicity of *P. graminis* seems rare, barberry apparently is primarily responsible for the origin of new forms. (Cooperative investigations between the Divisions of Cereal Crops and Diseases and Barberry Eradication, Bureau of Plant Industry, U. S. Department of Agriculture, and the Minnesota Agricultural Experiment Station.)

Bacterial stalk rot of sweet corn. A. R. Stanley and C. R. Orton.

A bacterial stalk rot of corn appeared epiphytotically in the Ohio Valley at Lakin, West Virginia, in 1930 and 1931. The pathogen was isolated from the following varieties in 1930: Aviator, Golden Acre, Golden Sunshine, Golden Coin, Golden Bantam, 60-Day Make Good, and Premo Extra Early, and in 1931 from Alpha, Aviator, Bantam Delicious, Earliest of All, Early Market, Gills' Early Market, Golden Bantam, Golden Bantam (14-row), Golden Bantam (15 x 16 hybrid), Golden Coin, Golden Crosby, Golden 60-Day Sweet, Golden Early Market, Golden Gem, Golden Rod, Golden Sunshine, Mayflower Extra Early, New Extra Early Bantam, Premo, Red-Leaved Early Bantam, Surprise, Burpee, Whipple's Early White, and Early Yellow. Artificial inoculations have been successful when the suscept is grown under sterile conditions on solidified Pfeffer's medium in 2 x 16 inch test tubes. Needle-prick inoculations on the stalk of young corn plants result in the parenchyma's being rapidly invaded, the tissues' turning watery brown and soft, and the stalks crumpling. The numerous strains are motile in broth cultures, produce H2S when grown on lead-acetate agar, and do not produce indol in tryptophane broth. The organism has been identified as Bacterium dissolvens and confirmed as such by Rosen. It shows a striking similarity to Bacillus carotovorus.

Sulphur barriers and graminaceous crop barriers to prevent spread of Phymatotrichum root rot. J. J. Taubenhaus and Walter N. Ezekiel.

Two types of barriers have been tested as possible means of preventing spread of Phymatotrichum root rot. Acid barriers were prepared by mixing 2 or 4 per cent of sulphur with the soil. These barriers were 4 to 6 inches wide and reached to the bottoms of containers or $4\frac{1}{2}$ feet deep in field plats. Cotton was planted on each side of the barriers; those plants on one side only were inoculated with Phymatotrichum. The inoculated plants succumbed, but the disease did not pass the sulphur barriers to attack the noninoculated plants on the other side. Neither the cotton roots nor the Phymatotrichum strands passed through the barriers. The soil within the barriers became as acid as pH 2.2, while that outside remained at the favorable pH of 7.5 to 8.

Single rows of graminaceous crops, such as sorghum and corn, tested in containers, were effective barriers. Cotton roots as well as Phymatotrichum strands failed to grow through the graminaceous root barriers, while roots of the latter grew out between the roots of the cotton plants but without contracting root rot.

Nursery plants as possible carriers of Phymatotrichum root rot. J. J. Taubenhaus and Walter N. Ezekiel.

Phymatotrichum root rot frequently appears on plantings in gardens, streets, or parks, where the disease was not known to occur before. In some such cases the disease originates from infected native vegetation or spreads from near-by infested areas. In other cases it has been suspected that root rot was brought in with the nursery plants. Balled and nonballed 2-year-old trees, from 2 nurseries where Phymatotrichum root rot had caused heavy damage, were set in cylinders and in the open, in a place where root rot had not occurred previously, although susceptible plants had been grown there constantly. Cotton was interplanted throughout. By midsummer, the cotton plants growing next to a balled hackberry tree in 1 cylinder succumbed to root rot. Likewise, a number of cotton plants growing next to 5 balled jujube plants planted in the open died from root rot only shortly before these jujube trees also succumbed. The presence of Phymatotrichum root rot in both cases resulted from the transmission of the disease on the roots or perhaps as sclerotia in the soil around these balled nursery plants.

An Actinomycete antagonistic to a Pythium root parasite of sugar cane. E. C. Tims.

A number of Actinomycetes isolated from sugar-cane soils in southwestern Louisiana and grown on artificial media have proved antagonistic to a parasitic sugar-cane Pythium. One of these cultures proved especially active and was used in later tests. When applied to sterilized soil previously inoculated with Pythium under greenhouse conditions, the Actinomycete reduced the amount of root rotting in young cane and corn plants, and when grown in a nutrient solution produced a toxic principle that inhibited the growth of the sugar-cane Pythium. Filtrates from which the organism had been removed were toxic to the Pythium when added to string-bean-agar plates. This shows that the Actinomycete produces a toxic principle rather than exhausts the nutrients in the medium and starves the Pythium. Tests showed that change in the hydrogen-ion concentration of the nutrient solution was not the inhibiting factor. This toxic principle is apparently partially destroyed by heat of sufficient duration.

Behavior of mosaic in certain sugar-cane varieties in Louisiana. E. C. Tims and C. W. Edgerton.

Mosaic-infected P. O. J. 213 and P. O. J. 228 cane, observed at Baton Rouge since 1926, continued to produce some healthy stalks in 1931. However, the proportion of such healthy shoots produced from mosaic-infected seed was less in 1931 than in previous years. The percentage of infection in field plantings of P. O. J. 213 at Baton Rouge still remains small, 2 to 5 per cent.

A very different condition, however, occurs in cane from Reserve, a plantation about 50 miles south of Baton Rouge. The natural infection in P. O. J. 213 runs as high as 50 to 60 per cent, and there is considerable infection in Co. 281 and Co. 290, which are practically disease free at Baton Rouge. Mosaic-infected stalks of P. O. J. 213 and Co. 281 from Reserve, planted at Baton Rouge, produced only diseased shoots, all of which have remained infected throughout 1931. Also, 100 stalks of each of these 2 varieties showing mosaic in early summer were tagged and observed until planting time in October. They all showed the disease in typical form in late October.

This difference in behavior of mosaic in the 2 different sections suggests that there may be 2 strains of the sugar-cane-mosaic virus present in Louisiana.

Controlling bottom rot of lettuce by dusting. G. R. TOWNSEND.

Bottom rot (*Rhizoctonia solani*) usually destroys 30 per cent of New York's muckgrown lettuce. Losses to individuals of 85 per cent are common during warm, humid periods. No resistant strains are known. Breeding for escapement, crop rotation, careful sanitation, special cultivation, mulch papering, and green manuring have all failed as control measures.

Extensive experiments the past 3 years with fungicides have shown only mercury compounds to be efficient. Calomel, mercuric oxide, and mercuric chloride all injured the lettuce severely. The fungicide giving the best control of bottom rot with no injury to the crop was an ethyl mercury phosphate (DuBay No. 738). The best rate of application was about 25 pounds per acre. One application made 2 weeks before harvest gave practically complete protection. In 54 field experiments during 1930 and 1931 hand applications reduced the average incidence of bottom rot from 27 to 6 per cent.

Traction dusters have been built recently that blow the dust under the lower leaves. In 62 fields, covering 30 acres thus treated this year, the average incidence of bottom rot was reduced from 22.6 to 2 per cent. The average increase in yield was 160 crates per acre. Increases of over 500 crates have been secured.

Helminthosporium sigmoideum, the conidial stage of Sclerotium oryzae. E. C. Tullis.

Sclerotium oryzae, which causes stem rot of rice, has been found to produce a conidial stage, identified as Helminthosporium sigmoideum. This conidial stage has been observed on agar cultures, on plants grown in vitro, and on plants grown in soil in the laboratory. Conidia of H. sigmoideum were found also in a herbarium specimen of S. oryzae collected in Italy by Briosi and Cavara. The genetic connection of the two stages has been demonstrated in inoculations and in cultural studies with both stages. Blue Rose rice seedlings grown aseptically on corn-meal agar in test tubes and inoculated with sclerotia of S. oryzae were killed by the fungus. Subsequently conidia of H. sigmoideum developed on these seedlings. These conidia germinated, infected rice seedlings, and produced in them and on agar the characteristic sclerotia of S. oryzae. The entire cycle, from sclerotia to mycelium to conidia to mycelium to sclerotia, has been followed under controlled conditions in the laboratory, and on plants grown aseptically on agar and in the soil. (Cooperative investigations between the Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, and the Arkansas Agricultural Experiment Station.)

Ophiobolus oryzinus on rice in Arkansas. E. C. Tullis.

Ophiobolus oryzinus, originally found in the Philippine Islands by C. F. Baker, on rotting rice straw, has been found to produce a disease of rice in Arkansas. In artificial inoculation experiments, O. oryzinus is pathogenic on Fortuna and Blue Rose rice plants in seedling and heading stages and on red rice plants in the seedling stage. Certain of the plants have been killed outright, while others have been injured through loss of leaf area. Red rice plants also have been found with their crowns invaded. Infected plants failed to tiller normally, producing tillers only after the heading of the first culm. Invasion of the host occurred by direct mycelial penetration of the epidermis of the basal leaves. Appressoria were formed and aided in the invasion of tissues. (Cooperative investigations between the Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, and the Arkansas Agricultural Experiment Station.)

Two seed-transmitted ring-spot diseases of tobacco. W. D. VALLEAU.

Two ring spots of tobacco, designated green and yellow, are present in Kentucky. In one, chlorotic patterns are in shades of green; in the other, shades of green and yellow, with frequently large yellow or nearly white leaf areas. Both viruses are transmitted through tobacco seed in percentages up to 15. Affected seedlings of yellow ring spot turn yellow soon after germination. Of 24,060 seedlings grown from plants affected with yellow ring spot, 1,175, or 4.9 per cent, were positive a few days after germination. Seed transmission of green ring spot is difficult to detect except at low temperatures (50 to 65° F.), when the edges of the leaves of affected plants become chlorotic to necrotic. Tobacco seed probably has not played a part in the distribution of yellow ring spot, as affected seedlings grow so slowly that they would never be set. It may have played a part in the distribution of green ring spot. The flower parts of affected plants appear normal, but a majority of the pollen grains are somewhat small and sterile. In the absence of other symptoms, ring spot may be diagnosed by a study of pollen. Ovule sterility varies greatly when self-pollination occurs, but this may be due to abortive pollen.

The development of Cercospora leaf spot on sugar beets grown under different cultural conditions. Edgar F. Vestal.

The percentage of occurrence of Cercospora leaf spot in rows spaced 22 inches apart was on August 30, with 12-inch blocking, disease-free 26.6, light infection 28.5, heavy infection 30.7, dead 14.4; with 20-inch blocking, disease-free 30.6, light infection 56.9, heavy infection 3.9, dead 8.4. On September 30 the percentage occurrence in 12-inch blocking was disease-free 28.2, light infection 4.8, heavy infection 16.8, dead 50.2; with 20-inch blocking, disease-free 29.6, light infection 6.7, heavy infection 29.6, dead 34.0.

The early reading shows nearly 8 times as many heavily infected and 4 more dead leaves among the 12-inch than among the 20-inch blocking. The later reading showed nearly ½ more dead leaves and half as many heavily infected leaves in the 12-inch blocking as compared with the 20-inch. The epidemic was delayed about 1 month by the wider spacing, affording better conditions for development of these beets. The yields were 13.5 and 15.4 tons per acre, respectively, for the 12- and 20-inch blocking. The sugar content and purity were nearly equal for both.

A similar comparison between the 24- and 16-inch spacings showed nearly the same results as above.

Decomposition of the safranin precipitate of mosaic virus of tobacco. CARL G. VINSON.

The safranin precipitate of the virus of tobacco mosaic has been satisfactorily decomposed by adding Lloyd's alkaloidal reagent to it. The mixture is agitated at intervals for about ½ hour, and then is centrifuged at high speed. One treatment with the reagent may suffice to remove all the safranin from the supernant liquid, but, if not, the treatment is repeated until all the dye is removed. After removal of the safranin the supernatant liquid is usually more highly infectious than the original juice from which the safranin precipitate is prepared. This method of purification is simple and rapid and involves only very mild treatment of the virus.

Comparison of juices from diseased and healthy tobacco plants. Carl G. Vinson and Edgar J. Gildehaus.

The virus of tobacco mosaic can be precipitated from juice of diseased plants by addition of a solution of lead acetate. After washing the precipitate with a M_3 solution of primary potassium ortho phosphate the virus can be freed from the precipitate by means of a dilute solution of potassium hydrogen phosphate, which is nearly neutral in reaction. Such a solution of the virus is very infectious and contains Kjeldahl nitrogen. The same procedure applied to juice from healthy plants yields a solution that contains no Kjeldahl nitrogen.

Gibberella moniliformis on corn plants in the field. R. K. Voorhees and A. H. Eddins.

Gibberella moniliformis, the perfect stage of Fusarium moniliforme, has been found occurring naturally on corn leaf sheaths in Florida. Macroscopically, the perithecia of this fungus are similar to those of G. saubinetii but are characteristically less cespitose on this host. Wineland reported the development of perithecia of G. moniliformis in pure culture. In the present investigations perithecia of the fungus developed in cultures from conidia of F. moniliforme. Perithecia also developed on cornstalks inoculated with single and multisporous conidial cultures. Cultures from single ascospores have produced both microconidia and macroconidia of F. moniliforme.

Particle size of the virus of tobacco mosaic in purified solutions. John G. Waugh and Carl G. Vinson.

Results of preliminary experiments indicate that the radius of the virus particle in our purified solutions is less than 5 millimicrons.

The toxicity of naphthalene for fungi of the Sclerotium rolfsii type. FREEMAN WEISS and E. L. EVINGER.

Naphthalene suspended in corn-meal agar in minute crystals is toxic to cultures of Sclerotium rolfsii grown on this medium. The germination of sclerotia and growth of mycelium are retarded by concentrations as low as 1 part of naphthalene to 10,000 (weight basis) of medium. A concentration of 1:5,000 entirely prevents growth. Sclerotia kept on agar plates of greater than 1:5,000 naphthalene concentration do not revive when transferred to naphthalene-free agar. Direct contact with naphthalene crystals is not requisite for toxic action, as naphthalene vapor kills sclerotia in 3 to 4 days' exposure at 30° C.

In preliminary tests with potted plants, and in field plots, repression of Sclerotium rolfsii by naphthalene, without toxic effects on the crop plants tested, is indicated. As a soil fungicide naphthalene possesses the additional features of exceptionally low cost (2 to 4 cents a pound) and effective distribution in soil because of its volatility. Preliminary trials with other soil-inhabiting fungi, such as Rhizoctonia, Fusarium, and Penicilium, indicate that naphthalene is somewhat fungicidal generally. Naphthalene already has an accepted standing as an acaricide and rodent repellent.

Control of celery mosaic by eradicating wild hosts. F. L. Wellman.

The celery mosaic that occurs in Florida is not seed-borne but persists from season to season in Commelina nudiflora and other wild hosts. Insects, chiefly Aphis gossypii, migrating from infected weeds to celery, can carry the mosaic virus at least 600 feet, though initial infection occurs more commonly within 50 feet of wild hosts. Four closely adjacent celery fields were used in the eradication experiments during the winter of 1930-31. In fields A and B weeds were removed from ditch banks and around buildings and bushes shortly after celery was transplanted. In field C eradication was practiced 2 months after it was begun on A and B; in field D no weeds were removed. In parts of field A there was more than 60 per cent of infection in 1929-30. In 1930-31 the disease occurrence in these same areas was reduced to around 6 per cent. In field B practically the same results were obtained. In fields C and D nearly as heavy infection occurred in 1930-31 as in 1929-30, though the somewhat cooler season of 1930-31 seemed to inhibit its appearance to a considerable extent.

Wilt resistance of Pride of Muscatine (K-S4) watermelons on heavily versus moderately infested soil. J. J. WILSON.

A comparison was secured in 1931 of the response of the wilt-resistant variety, Pride of Muscatine (K-S4), in 2 fields infested with Fusarium niveum. Field No. 1 had been cropped to melons 6 consecutive years, while No. 2 had not. On July 3, Pride of Muscatine showed 25 per cent wilt in field No. 1 and 3.5 per cent in field No. 2. Stand counts of plants were continued until October 5, at which time 43 per cent had died in field No. 1 and 11 per cent in field No. 2. The variety Kleckley Sweet, used as checks, showed 68 per cent wilt on July 3, when the plants had to be sacrificed to prevent cross pollination. The response of Pride of Muscatine in field No. 2 was typical of the performance of this variety in 1931 when grown on 750 acres of wilt-infested soil in Muscatine County.

During the first 2 weeks following emergence it was noted that 17 per cent of the Pride of Muscatine plants and 31 per cent of the Kleckley Sweet plants had died in field No. 1. This heavy mortality of seedlings has occurred during the past 5 years in 3 experimental fields, continuously cropped to melons, where the soil is very heavily infested with Fusarium niveum.

Ampelomyces quisqualis on clover mildew. CECIL E. YARWOOD.

During the late summer and fall of 1931, Ampelonyces quisqualis, a fungous parasite of powdery mildews, was apparently responsible for the almost complete killing out of clover mildew in experimental plots of red clover. It also was abundant on other mildews and has been isolated in pure culture from the following hosts: Erysiphe polygoni on red clover, Microsphaera alni vacinii on catalpa, Sphaerotheca pannosa on rose, Erysiphe cichoracearum on zinnia, E. cichoracearum on Ambrosia artemisiifolia, Microsphaera alni on Lonicera sp., and an undetermined mildew species on Plantago major. The isolations from the first five hosts show distinguishing cultural characteristics on agar. Inoculations were performed by atomizing a suspension of Ampelomyces spores onto vigorously growing mildew colonies. Infection of clover mildew has resulted from inoculation with cultures from clover, catalpa, and rose mildews. The optimum temperature for spore germination of Ampelomyces and for infection of clover mildew is about 25° C. Pycnidia are produced in abundance in about 6 days following inoculation of living mildew, but growth and pycnidium formation are very slow in agar cultures. At 25° C. growth and conidium formation of clover mildew colonies may be completely stopped in about 8 days after inoculation with Ampelomyces. (Cooperative investigations, Division of Forage Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, and Purdue University Agricultural Experiment Station.)

Reversible phototropism of the germ tubes of clover powdery mildew. CECIL E. YARWOOD.

In high light intensities, such as full exposure to day-light, even of a cloudy day, the tubes of powdery mildew of red clover are positively phototropic. In relatively low light intensities they are negatively phototropic. Appressoria are produced by the germ tubes due to contact stimuli, their formation is dependent to certain extent on the vigor of the phototropism, and they may be formed by positively- or negatively-phototropic germ tubes. The percentage of germination is much higher in light than in darkness, but good infection is secured on the host, either in light or in darkness. (Cooperative investigations, Division of Forage Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, and Purdue University Agricultural Experiment Station.)

Lubricating-oil sprays and their effect on potatoes. Paul A. Young.

Oil sprays control potato aphids cheaply. In a field 103 hills of Triumph potatoes, sprayed with a 2 per cent cresoap emulsion of a 90 per cent non sulphonatable oil, yielded 9.1 per cent less than the checks; 55 other hills sprayed with a 1 per cent emulsion of this oil yielded 5 per cent more than the checks. These differences were insignificant. Similar vines sprayed with 8 to 16 per cent oil emulsions produced few tubers and the vines died prematurely. Pure oils and 4 to 16 per cent emulsions caused the following symptoms in potato leaves: translucent spots; black to brown spots; epiphyllous white spots normal or brown hypophyllously; epiphyllous blackening and browning of veins; and rolling, wilting, and yellowing. Oils injured young leaves more than old ones. Oil partially girdled the bases of potato stems, causing some aerial tubers. N-decane quickly caused translucent and brown leaf spots. One to 8 per cent cresoap emulsions freeze, ice crystals forming between layers of oil and air. Blocks and lamellae of ice form between oil layers and networks. Strong cresoap emulsions reform in melting. Cresoap miscible oil, touching water, instantly formed emulsified oil droplets 0.2 to 3 μ in diameter.



SOME NUTRITIONAL DISORDERS IN CORN GROWN IN SAND CULTURES¹

N. A. PETTINGER, R. G. HENDERSON, AND S. A. WINGARD²

INTRODUCTION

During the course of some sand-culture experiments with corn in the summer of 1930, there occurred several different types of disorders in the plants that have since been traced to nutritional causes. Chief among these were three distinct types of chlorosis, two of which appear to be different from the several types previously reported in the literature for corn. It was noticed also that the plants were developing sterile tassels and, therefore, would not be able to reproduce normally. These abnormal developments in the leaves and tassels led to an investigation of their causes during the fall of 1930 and summer of 1931, and the present paper reports the results obtained in these studies.

Disorders in plants usually are classified under three types, namely: (1) hereditary, (2) infectious, and (3) nutritional. The corn used in the studies here reported was an F_1 cross of two inbred strains of the Reid Yellow Dent variety grown at Bloomington, Ill. The F_1 , as well as the parental strains, has never developed chlorosis or been sterile when grown in soil, which indicates that the maladies observed in the sand cultures were not of the hereditary type. On some of the leaves, the chlorotic areas appeared to be harboring pathogenic organisms, which suggested that the trouble might be of an infectious nature. There was also the possibility that the nutrient solutions applied to the sand were either deficient or toxic in one or more of the nutrients required by the plant or that the nutrients were not applied in the correct proportions with respect to each other. In attempting to learn the cause or causes of these disorders, therefore, it was necessary to attack the problem from both the infectious and nutritional standpoints.

DESCRIPTION OF DISORDERS

Types of chlorosis: The leaves of the plants grown in the sand cultures were not uniform with respect to their chlorophyll deficiencies. Casual observation of the chlorotic areas showed the presence of two distinctly different types of chlorosis, but closer observation and study of one of these

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types disclosed minor differences in the outlines of the chlorotic areas, which indicated that there were perhaps three types present. This suspicion was confirmed by the fact that the differences observed in the leaves have now been traced to different causes. To facilitate reference to these different types of chlorosis, they will henceforth be designated in this paper as types A, B, and C.

Type A appears to be identical to that described by Jones (5), which was found to be caused by a deficiency of magnesia. The affected areas

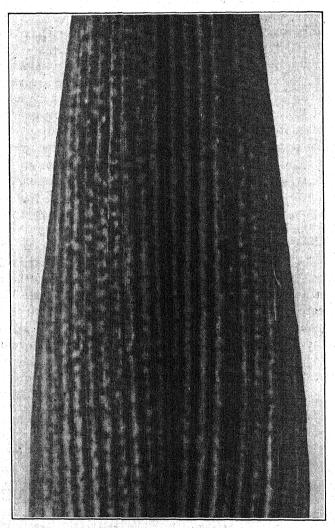


Fig. 1. Leaf from corn plant grown in sand culture that was deficient in magnesuim, showing type-A chlorosis.

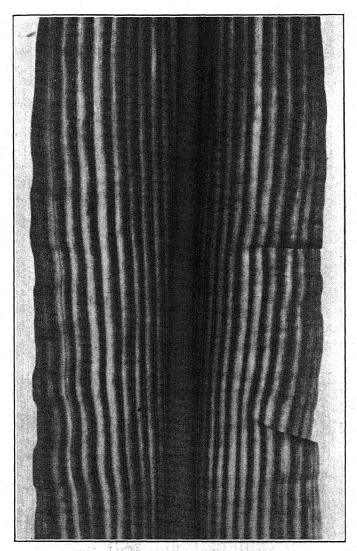


Fig. 2. Leaf from corn plant grown in sand culture that received an excess of sodium, showing type-B chlorosis.

appeared as long, narrow, chlorotic streaks that are more or less continuous from the base to the tip of the leaves. The streaks, however, are very irregular along the margins and in some cases are broken up into chains of alternate green and chlorotic splotches (Fig. 1). In contrast to this, the streaks in type B are continuous, with very regular margins, and extend from vein to vein the full length of the leaf (Fig. 2). In the early stages

of growth of the plants, these types of chlorosis were characterized by alternate streaks of light green and dark green in the leaves; the light green being in the intervascular tissues and the dark green in the vascular regions. As growth progressed, the light green of the intervascular tissues was gradually lost, making the chlorotic streaks more apparent. In the advanced stages of development of type A necrosis set in, causing the central parts of the chlorotic streaks to become brown.

Type C was quite distinct in appearance from either A or B and could readily be distinguished from them by a casual observer. This type had the appearance of a bacterial leaf spot and was thought at first to be of bacterial origin. It is also similar to a hereditary type of chlorosis described by Koehler and Holbert (6) and designated as "dying between the veins." The white or chlorotic spots that characterized this trouble first appeared on the leaves when the plants were about 3 weeks old. At this time the affected areas were small, but they enlarged as the leaves grew. At all times, however, they were confined to the tissues between the veins. The

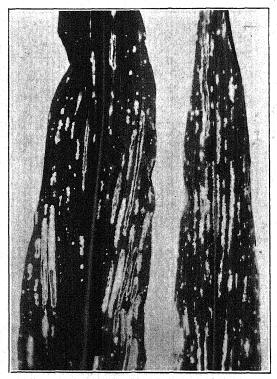
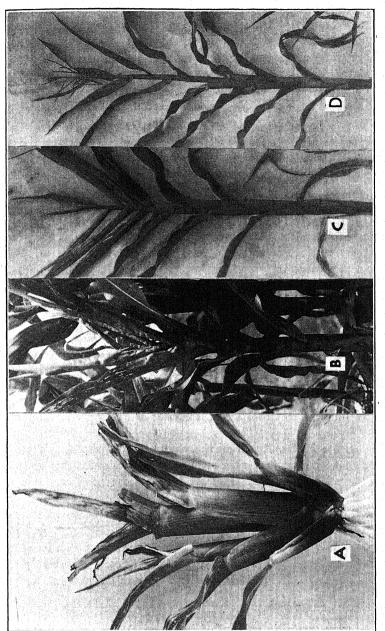


Fig. 3. Leaves from corn plants grown in sand cultures that were deficient in minor elements, showing type-C chlorosis, which is attributed to a deficiency of manganese.



Abnormal developments in corn plants grown in sand cultures that received no minor elements. A. Affected in seedling stage. B and C. Affected just prior to tasseling. Note normal growth made during earlier stages. D. Plant that developed a trace of chlorosis at tasseling time. Fig. 4.

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green pigment disappeared very rapidly and completely, so that all of the affected areas appeared translucent from the beginning. These chlorotic areas were irregular in outline and more or less longitudinal in shape. When abundant in the young leaves they elongated to such an extent as to coalesce, thereby forming long, continuous chlorotic streaks in the more advanced stages (Fig. 3 and Fig. 6, A). When these areas appeared at some distance from each other, they often failed to coalesce on enlargement, giving the leaf a "buck-shot" appearance. As the season progressed the chlorotic tissues began to break down and ceased to function in the activities of the plant (Fig. 3). This resulted in streaks of dead, brown tissue in the center of the chlorotic areas. The necrotic tissue sometimes fell away, leaving holes in the leaves. In the more severe cases the chlorotic areas were covered by a clear gelatinous substance.

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Dwarfing: All types of chlorosis were accompanied by reduced growth. The plants affected with types A and B were only slightly shorter, the internodes and leaves retaining their normal proportions throughout the plant. In type C, however, varying degrees of distortion of growth occurred, depending on the stage of growth in which the plant became affected. When affected in the early seedling stage the growing points were either injured or killed, resulting in stunted and deformed stalks and leaves, the leaves being only ragged vestiges a few inches in length (Fig. 4, A). Such plants also showed a tendency to tiller. In plants in which the cause of the disorders did not become acute until the later stages of growth, the lower parts of the plant developed normally but the upper leaves and internodes were malformed. The upper internodes were very much shortened, giving the leaves a bunched appearance (Fig. 4, B, and C). The leaves also showed a tendency to curl inwardly at the margins and were usually very chlorotic.

Sterility: In the 1930 cultures all the plants that reached the shooting and tasseling stage before the experiment was discontinued proved to be sterile. In the milder cases of chlorosis tassels were produced but the flowers were sterile (Fig. 4, D), while in severe cases no tassels or shoots whatever developed (Fig. 4, B, C). The latter condition was associated more often with the type-C chlorosis than with either of the other types. Plants that were only slightly chlorotic with stalks and leaves of apparently normal proportions developed tassels and shoots that might have been functional had the plants been allowed to remain longer in culture.

CONDITIONS UNDER WHICH THE CORN WAS GROWN

The plants were grown in galvanized iron cans, 18 in. in diameter and 24 in. deep, filled with quartz sand to within an inch of the top. The cans

were coated with paraffin on the inside to prevent zinc toxicity and were provided with drainage at the bottom. Insulation against air temperatures was attained by inserting the cans in a long wooden box 18 in. above the ground and filling in around them with soil. Eight kernels were planted in each can and the seedlings thinned down to 4 when a week old. The 1930 cultures were planted on June 6 and terminated during the second week of August, while the 1931 cultures were planted on May 18 and terminated during the first week of August. At the time the experiment was terminated in both years the plants were beginning to form shoots and tassels. The experiment was conducted in an open field.

The purpose of the sand-culture tests herein described was to determine the effect of different concentrations of the nutrients supplied to the sand on the composition of certain plant parts. The plan was to vary one element at a time, keeping all other elements constant in the cans in which the element in question varied in concentration. Thus, in the cans where calcium was applied in different concentrations, magnesium, nitrogen, phosphorus, potassium, iron, and sulphur were applied at their respective concentrations to all cans throughout the experiment. Five elements were varied in this manner, namely, magnesium, calcium, nitrogen, phosphorus, and potassium. Each of these elements, in turn, was applied in 5 different concentrations, making a total of 25 different nutrient cultures. The first concentration was made "very low," the second "low," also, but higher than the first, the third "medium," the fourth "high," and the fifth "very high." The medium concentration, which was used for a standard, was based (with slight modifications) on the nutrient solutions used by Duley and Miller (2) in similar work at the Missouri station. Their solution was adopted as a standard because of the success they had in securing good Each solution was tested in duplicate.

The concentration of each of the stock nutrient solutions used is as follows:

$MgSO_4 \cdot 7H_2O$								
Ca(NO ₃) ₂ ·4H ₂ O	250		"	"	"	"		
NaNO ₃	136.7		44	"		"		"
KCl								
CaCl ₂ ·2H ₂ O	140	"	"	"	"	"	"	"
KH ₂ PO ₄	50		"	"	"	"	"	66
NaH ₂ PO ₄ · H ₂ O	50		"	"		6,6	"	"
FeCl ₃ ·6H ₂ O	8			"	"	"	"	"

The quantities of each stock solution applied to the different cultures in 1930 are given in table 1. The same quantities of these solutions were added in 1931, and, in addition, boron, zinc, copper, and arsenic were added at the rate of 0.5 part per million and manganese at the rate of 1.0 p.p.m. The

TABLE 1.—Cubic centimeters of stock nutrient solutions added to 4 liters of water

	Cans 1 and 2	Cans 3 and 4	Cans 5 and 6	Cans 7 and 8	Cans 9 and 10	
		Magne	esium Series			
$\begin{array}{c} \text{MgSO}_4 \cdot 7\text{H}_2\text{O} \\ \text{Ca} \left(\text{NO}_3 \right)_2 \cdot 4\text{H}_2\text{O} \\ \text{KCl} \\ \text{KH}_2\text{PO}_4 \\ \text{FeCl}_3 \cdot 6\text{H}_2\text{O} \end{array}$	1 20 20 20 20 2	5 20 20 20 20 2	10 20 20 20 20 2	15 20 20 20 20 2	20 20 20 20 20 2	
		Calc	ium Series			
MgSO ₄ · 7H ₂ O Ca(NO ₃) ₂ · 4H ₂ O NaNO ₃ KCl CaCl ₂ · 2H ₂ O KH ₂ PO ₄ FeCl ₃ · 6H ₂ O	10 4 32 20 0 20 20	10 10 20 20 0 20 20	10 20 0 20 0 20 20 20 2	10 20 0 20 20 20 20	10 20 0 20 60 20 2	
		Nitr	ogen Series			
$\begin{array}{cccccccccccccccccccccccccccccccccccc$	10 4 0 20 16 20 2	10 10 0 20 10 20 2	10 20 0 20 0 20 20 20	10 20 40 20 0 20 20	10 20 120 20 0 20 20 2	
		Phosy	ohorus Series			
$\begin{array}{c} MgSO_4 \cdot 7H_2O \\ Ca(NO_3)_2 \cdot 4H_2O \\ KCl \\ KH_2PO_4 \\ NaH_2PO_4 \cdot H_2O \\ FeCl_3 \cdot 6H_2O \\ \end{array}$	10 20 40 2 0 2	10 20 31 10 0 2	10 20 20 20 20 0 2	10 20 20 20 20 10 2	10 20 20 20 20 20 20	
	sa., 1	Pota	ssium Series			
$\begin{array}{c} MgSO_4 \cdot 7H_2O \\ Ca (NO_3)_2 \cdot 4H_2O \\ KCl \\ KH_2PO_4 \\ NaH_2PO_4 \cdot H_2O \\ FeCl_3 \cdot 6H_2O \\ \end{array}$	10 20 4 4 16 2	10 20 10 10 10 2	10 20 20 20 20 0 2	10 20 80 20 0 2	10 20 160 20 0 2	

compounds in which these elements were added are listed in table 3. In 1931 an attempt was also made to raise the nitrogen level of the experiment by adding 10 cc. of sodium nitrate to all cultures except those made deficient in nitrogen. After 3 applications, however, this practice was discontinued because of the appearance of type B chlorosis already described.

When it was desired to add nutrients to the sand the specified amounts of the various stock solutions were first mixed together in a beaker, then added to 4 liters of distilled water, and sprinkled over the surface of the sand. The chemicals used were of the usual "C. P." grade.

HYPOTHESES TO ACCOUNT FOR THE DISORDERS

When the type-C chlorosis was first observed, it was thought to be a bacterial trouble. This was indicated by the presence of a gelatinous substance on the surface of the chlorotic areas, which resembled the exudate common to many bacterial infections on plants. Microscopic examination showed that this material contained numerous bacteria. Isolations yielded two types of bacterial colonies, one of which was bright yellow, the other cream-color. Both organisms, however, proved to be noninfectious on healthy corn grown in soil in the greenhouse. Repeated inoculations by needle punctures and swabbing of the leaf surfaces gave negative results. Similar inoculations made directly from infusions of the affected tissues in nutrient broth also gave negative results, showing conclusively that the bacteria present in the chlorotic areas were only secondary. A careful examination of the roots of the affected plants showed them to be free from bacterial and fungous infection. In contrast to the type-C chlorosis, types A and B did not appear to be of an infectious nature. Furthermore, the sterility and stunting showed no evidence of having been caused by pathogenic organisms.

After eliminating the possibility that these disorders might be of either hereditary or pathogenic origin, it became necessary to consider them from the nutritional standpoint. This naturally led to a close examination of all the cultures for possible relations between the presence and severity of the chloroses and other disorders and the nutrients applied to the sand.

Chloroses: It was found that the type-A chlorosis (Fig. 1) occurred only in the cultures that received insufficient magnesium. Cans 1 and 2 (table 1) of the magnesium series received very small amounts of magnesium and supplied the excellent specimen shown in figure 1. The plants grown in cans 3 and 4 of this series were nearly normal in color; the intervascular tissues of the leaves were light-green but were not so devoid of chlorophyll as those of cans 1 and 2. Since this type of chlorosis occurred only in the magnesium-deficient cultures, it is apparent that the deficiency of magnesium was the cause of this type of chlorosis.

The type-B chlorosis was found to occur in all cultures where sodium was added in the nutrient solutions. In the calcium-deficient cultures of the calcium series sodium was added as sodium nitrate to replace the nitrate lost by withholding the calcium of calcium nitrate. In the nitrogen series this type of chlorosis appeared in the cultures that received an excess of nitrates, the nitrate over and above the basal amount of calcium nitrate being added as sodium nitrate. In the excess-phosphate cultures sodium was added as sodium di-hydrogen phosphate to increase the phosphate above that supplied by the basal quantity of potassium di-hydrogen phosphate. Sodium was also added as sodium di-hydrogen phosphate in the potassium series to replace the phosphate lost when potassium di-hydrogen phosphate was withheld to make potassium subnormal in the potassium-deficient cultures. All of these cultures showed the smooth-margin, continuous streaking shown in figure 2 and indicate that this type of chlorosis was caused by an excess of sodium in these cultures. This belief is strengthened by the observation that when an attempt was made to raise the nitrogen level of the experiment by adding 10 cc. of the sodium-nitrate stock solution to all cultures except those deficient in nitrogen, this same type of chlorosis made its appearance in a mild form in nearly all of the cultures. Hence, after three successive additions of nutrients with the sodium nitrate included, this practice was discontinued. The plants gradually regained a normal green color in the intervascular tissues of the leaves, which confirms the belief expressed above that this type of disorder resulted from sodium toxicity.

The type-C chlorosis (Fig. 3) occurred throughout all of the 1930 cultures except those that received an excess of calcium or a deficiency of nitrogen. Occasionally single plants in other cultures were free from this type of chlorosis, even though other plants in the same culture were affected.

The fact that the excess-calcium and nitrogen-deficient cultures were free from the type-C chlorosis suggests the possibility that a high-calcium content or a low-nitrogen content in the nutrient solutions might explain the lack of chlorosis in these cultures. However, a close study of the solutions applied to the other cultures of the experiment shows that neither of these situations can explain the apparently normal cultures. On the other hand, there is a close correlation between the ratio of calcium to nitrogen and the presence or absence of chlorosis in the leaves; the normal cultures having ratios above 1.5:1 and the chlorotic ones ratios of 1.5:1 or lower. This indicates that the ratio between these elements may have been of more importance than the actual amounts added. In what way a wide Ca:N ratio might operate to prevent chlorosis is not apparent at present; the close correlation, however, may be significant.

Brown (1) has reported chlorosis in wheat grown in sand cultures in which calcium nitrate and potassium di-hydrogen phosphate were applied

TABLE 2.—Effect of nutrient ratios on the development of chlorophyll in the leaves of corn plants grown in sand cultures

	Chlorosis	Present '' Trace Absent '' Character Absent '' '' '' '' '' '' '' '' ''
	${ m Ca(NO_3)_2}\colon { m KH_2PO_4}$	
Ratio of	Ca: N	
	Ca: Mg	0.00 0.00
	Potassium	Medium
tion	Phosphorus	Medium
Nutrient concentration	Nitrogen	Medium ;; ;; ;; ;; ;; ;; Very low Low Medium High Very high Medium ;; ;; ;; ;; ;; ;; ;; ;; ;; ;; ;; ;; ;;
Nut	Calcium	Medium (
	Magnesium	Very low Low Medium High Very high Medium (((((((((((((((((((

in approximately equal molecular proportions. He states that this was caused by nutrient balance and increased permeability. That the disorders observed by the writers were not due to this cause is shown by the $\mathrm{KH_2PO_4:Ca(NO_3)_2}$ ratios given in table 2. Nor do the Ca: Mg ratios (Table 2) explain the condition of any of the cultures. The presence of the type-A chlorosis in the magnesium-deficient cultures is believed to be the result of insufficient magnesium rather than an unfavorable Ca: Mg ratio. The evidence indicates that in the case of calcium and magnesium the amounts added were of more importance than the ratio between these elements.

Table 1 shows that the chlorosis-free cultures of 1930 were the only ones that received calcium chloride in the nutrient solutions. This material was added to cultures in the calcium series to build up an excess of calcium and in the nitrogen series to make up for the loss of calcium occasioned by withholding calcium nitrate from the nitrogen-deficient cultures. That the calcium thus added does not account for the lack of chlorosis has been pointed out above. It is also unlikely that the additional chlorine added in the calcium chloride prevented the development of chlorosis, for chlorine also was made high in the potassium series by adding additional potassium chloride to the excess-potassium cultures. These cultures, in spite of the high chlorine content of their nutrient solutions, were as chlorotic as most of the others.

Since the evidence just presented concerning some of the major plantfood elements is more or less ineffective in explaining the occurrence of the type-C chlorosis in the 1930 cultures, the writers turned their attention to a consideration of the so-called minor elements, manganese, zinc, copper, arsenic, boron, etc. These elements were omitted because it was believed that sufficient amounts of each would be included as impurities in the other chemicals and sand to supply the needs of the plants. However, when the chlorosis made its appearance, it was decided to start additional cultures to determine whether the lack of minor elements might be responsible for the These tests were begun in the greenhouse in September, 1930. chlorosis. Corn from the same lot of seed as was used in the field cultures was planted in the same kind of sand. The cultures remained in the greenhouse until after the seed had germinated. They were then placed outside, so that the plants would secure better light for growth. At the age of 3 weeks, the seedlings began to show chlorophyll breakdown. Sixteen of them were then transplanted individually to 3-gal. earthenware pots filled with sand. Onehalf of these pots were then supplied with the basal nutrient solution only, while the others were given the minor elements listed in table 3, in addition to the basal nutrients. Several seedlings also were transplanted to pots filled with a fertile loam soil.

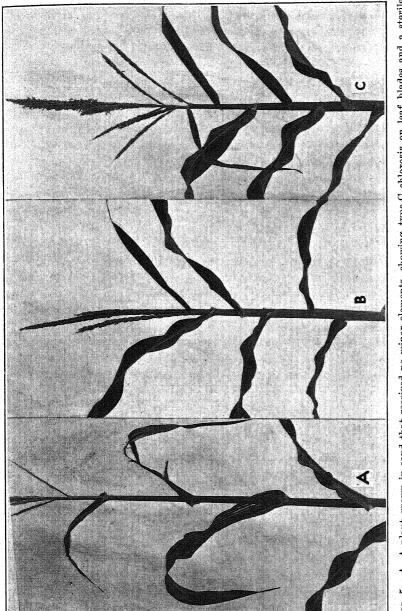


Fig. 5. A. A plant grown in sand that received no minor elements, showing type-C chlorosis on leaf blades and a sterile tassel. B. Grown in sand without minor elements until chlorosis began to appear, then transplanted to sand supplied with minor elements. C. Grown in sand without minor elements until chlorosis began to appear, then transplanted to a fertile loam soil. Note the freedom from chlorosis and the presence of fertile tassels in B and C.

The results of this test indicate that the chlorosis observed in the field cultures and reproduced in the greenhouse cultures was caused by a lack of one or more of the minor elements. Representative plants of the sand and soil cultures are shown in figure 5, and leaves of the same plants are shown in figure 6. The cultures that received basal nutrients only were

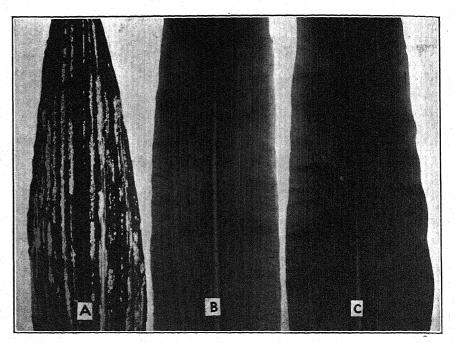


Fig. 6. A, B and C. Leaves from plants shown in figure 5 A, B and C, respectively.

uniformly chlorotic (Fig. 5, A; Fig. 6, A), while those that received the minor elements, in addition to the basal nutrients, were free from chlorosis (Fig. 5, B; Fig. 6, B). The plants grown in soil were also free from any chlorophyll defects (Fig. 5, C; Fig. 6, C) and were a darker green than those grown in sand either with or without the minor elements.

Owing to the pressure of other work, it was impossible to extend these tests to determine which of the five minor elements added were effective in preventing the chlorosis. Judging from information available concerning the functions of zinc, copper, arsenic, and boron in plants, it is improbable that any of these elements prevented the development of chlorosis. Concerning manganese, however, there is considerable evidence in the literature to indicate that the chlorosis observed in the sand cultures described here may have been due to a lack of sufficient available manganese. Thus, Mc-Hargue (8, 9) found that a lack of sufficient manganese caused chlorosis in

a number of economic plants and concluded that manganese plays a rôle of importance equal to that of iron in the synthesis of chlorophyll and carbon assimilation. Lee and McHargue (7) have recently described a type of chlorosis occurring in sugar cane grown in Hawaii found to be due to manganese deficiency. They successfully corrected the disease by applying manganous sulphate to the leaves. Gilbert, McLean, and Hardin (3) have been successful in correcting chlorosis in spinach by applying manganese solutions to the leaves. Skinner and Ruprecht (10) report that crops growing in certain soils of the Florida Everglades are generally chlorotic unless manganese is applied in a soluble form. These investigations, as well as others that might be cited, show quite conclusively that manganese is essential to chlorophyll formation.

That the occurrence of the type-C chlorosis in the 1930 cultures resulted from a deficiency of manganese and that the correction of this type of chlorosis in the sand cultures in the greenhouse was due to the addition of manganese rather than boron, zinc, copper, or arsenic were definitely shown by the 1931 sand cultures in the field. Since the greenhouse tests had indicated that the addition of the minor elements were effective in preventing the type-C chlorosis, the 1931 field cultures were all supplied with the

TABLE 3.—Composition of stock solutions of minor elements and amounts added to nutrient solutions applied to sand cultures

Chemicals used	Amount dissolved in 2 liters of water (grams)	Amount of stock solution added to 4 liters of water (cc.)	
As ₂ O ₃	0.528	10	
$ZnSO_4 \cdot 7H_2O$	1.76	10	
MnSO ₄ · 4H ₂ O	1.62	10	
H ₃ BO ₃	2.25	10	
CuSO ₄ · 5H ₂ O	1.572	10	

amounts indicated in table 3 at each application of nutrients. When the plants were about a month old, however, many of them began to show the development of typical spots of the type-C chlorosis. Acting on the theory that manganese was deficient rather than any of the other minor elements added, the manganese ration was increased tenfold, from 0.5 to 5.0 parts per million. After two applications at this rate the ration was reduced to 1.0 part per million for the remainder of the experiment. This treatment not only restored chlorophyll to the type-C chlorotic areas that had already appeared but prevented any further development of this type of chlorosis,

which clearly shows that a deficiency of manganese was the cause of this type of chlorosis and that manganese, rather than zinc, boron, copper, or arsenic, was the corrective agent both in the greenhouse cultures and those conducted in the field in 1931. The evidence also shows that 0.5 p.p.m. of manganese was insufficient to prevent chlorosis entirely but that 1.0 p.p.m. was adequate.

It will be recalled that the excess-calcium and the nitrogen-deficient cultures of the 1930 experiment were free from chlorosis, even though no minor elements were added. As already pointed out, these cultures were the only ones that received calcium chloride; hence, there is a possibility that the calcium chloride might have introduced small quantities of certain minor elements into these cultures and thus prevented the development of chlorosis. The manufacturer's analysis, which accompanied the calcium chloride, was as follows: "Mg and alkalies, 0.20%; BaO, -.001%; Na, trace; Fe, -.001%; SO₃, .01%." In the term "Mg and alkalies," traces of such metals as magnesium, potassium, strontium, and lithium might reasonably be expected to occur. It is doubtful, however, that any appreciable amounts of manganese would be present.

It is unlikely also that the calcium chloride had any effect on the availability of other materials by changing the reaction of the nutrient medium, because the calcium chloride stock solution was practically neutral in reaction and its addition in any amount should have had no effect on the reaction of the nutrient solution. The cultures that were free from chlorosis and also those receiving all elements in medium amounts were tested for reaction and found to have pH values of either 4.6 or 4.7. The solutions, therefore, were sufficiently acid to make available such materials as manganese, aluminum, iron, etc., had they been present. This precludes the likelihood of a lime-induced chlorosis, especially since chlorosis was absent in the excess-calcium cultures. It is unlikely also that the chlorosis resulted from the high acidity making available such quantities of manganese, aluminum, and iron as to be toxic, for these elements were not applied to the sand and, therefore, were not present in toxic amounts, even if totally available.

Sterility and dwarfing: As stated above, the tassels produced by the plants grown in sand cultures in the field in 1930 were mostly sterile (Fig. 4, B, C). These cultures, it will be recalled, received no minor elements. The plants grown in the greenhouse tests in sand that received no minor elements also produced sterile tassels (Fig. 5, A). However, the plants grown in soil (Fig. 5, C) and those grown in sand to which the minor elements were added (Fig. 5, B) produced fertile tassels. These observations indicate that one or more of the five minor elements added functioned

in the development of fertile staminate flowers in these plants. This was confirmed by the fact that there was no evidence of sterility in any of the 1931 cultures. All of these cultures received minor elements and developed functional flowers in both tassels and shoots. Furthermore, the 1931 cultures showed no evidence of the types of dwarfing (Fig. 4) found in the 1930 cultures. The general height of the plants grown in 1930 was only 40 to 50 in., whereas, in 1931, most of the plants ranged between 80 and 100 in. in height.

The studies of other investigators indicate that all of the sterility and much of the dwarfing observed in the 1930 cultures were due to a lack of boron. Thus, Warington (12) found that a deficiency of boron caused death of the growing apices in the broad bean. Johnston and Dore (4) report that the terminal shoots of tomato die when boron is excluded from the growing medium, and Swanback (11) states that one of the most apparent symptoms of boron deficiency in tobacco is the death of the terminal bud. There can be little doubt, however, that the lack of other minor elements also had some effect in reducing the general growth of the plants.

RESISTANCE TO FROST

In connection with the greenhouse tests an interesting observation was made on the effect of the minor elements on the ability of the plants to resist injury from frost. In starting the greenhouse tests to determine the effect of minor elements on chlorophyll development, 6 cans filled with sand were planted with 12 kernels each. When it was found later that this procedure furnished more plants than were needed for transplanting in the chlorosis studies, 3 cans were placed outside the greenhouse and allowed to remain there. One of these received an application of minor elements on September 22 (age, about 3 weeks) and, again, on September 26, in addition to the basal nutrients. A second can received but one application of minor elements, in addition to the basal nutrients, on September 26. The third can received only the basal nutrients and, therefore, served as a check on the effects of the minor elements.

During the night of September 30 there was a heavy frost, the temperature dropping to 34° F. The following day it became apparent that the minor elements had increased the resistance of the young plants to injury from frost. The cans were then moved into the greenhouse to prevent further injury from cold and were supplied with basal nutrients for 3 weeks more. They were photographed on October 20 and are reproduced in figure 7. The plants that received basal nutrients only proved to be very susceptible to frost and were severely injured (Fig. 7, A). The leaves were almost entirely killed and the stalks made little growth during the

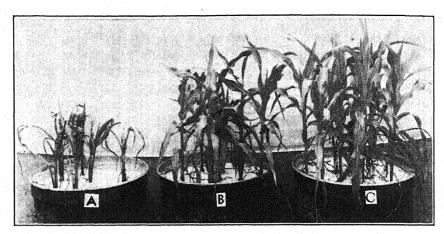


Fig. 7. Plants showing effect of additions of minor elements on resistance to injury from frost. A. Received basal nutrients only. B. Received one application of minor elements in addition to basal nutrients. C. Received two applications of minor elements in addition to basal nutrients.

3-week period following the frost. The plants that received only one application of minor elements were but slightly injured (Fig. 7, B). The tips of some of the leaves were killed, but the injury was not severe enough to stunt the plants much during subsequent growth. The plants that received two applications of minor elements showed no evidence of injury from the frost (Fig. 7, C) and made more growth than any of the other cultures. It is very evident from these results that the addition of minor elements enabled the young corn plants to withstand low temperatures much better than where the basal nutrients only were supplied.

SUMMARY

This paper deals with the description, cause, and prevention of disorders that occurred in corn plants grown in pure quartz sand supplied with basal nutrients only. The disorders consisted of three distinct types of chlorosis in the leaves, sterility of the flowers, and a dwarfing of the plants.

The three types of chlorosis have been designated as A, B, and C. Types A and B appear as continuous chlorotic streaks extending from the base to the tip of the leaves. The distinguishing feature between them is that in type A the streaks have ragged or irregular margins, while those of type B have smooth margins. Type C is very different from either A or B, being characterized by discontinuous streaking produced by chlorotic areas distributed at random in the intervascular tissues. Illustrations of these types of chlorosis are given in the text.

Field studies, isolations, and inoculations showed that the disorders observed were not of hereditary or pathogenic origin.

A number of nutritional relations are considered and discussed. The evidence presented shows that the type-A chlorosis was caused by a deficiency of magnesium, type-B by an excess of sodium, and type-C by a deficiency of manganese. Sterility of the reproductive organs is attributed to a deficiency of boron.

It was found that the addition of manganese, zinc, copper, boron, and arsenic to the basal nutrient solution increased the frost resistance of young corn plants grown in sand cultures.

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CHLOROSES OF THE ROSE

RICHARD P. WHITE

In the fall of 1927 a mosaic-like disease of roses growing under glass was observed in New Jersey, and since that time various surveys have shown this disease to be widespread throughout the United States. The symptoms of rose mosaic are very variable, depend on environmental conditions for expression, and have been reported as not clearly distinguishable from chloroses due to environmental effects and insect injuries. As a result, considerable confusion and doubt have arisen in regard to the nature of this disease.

It is the purpose of this paper to compare various types of chloroses of the rose and to present results of further experiments on the artificial transfer of rose mosaic to species and varieties of rose used as understock.

LITERATURE REVIEW

In 1909, 1910, and 1914 reports were received by the Plant Disease Survey from J. B. S. Norton in Maryland¹ of a mosaic-like mottling of rose. Norton was inclined to attribute these symptoms, however, to environmental conditions, especially excess soil moisture. Taubenhaus, in 1923² and 1925,³ reported from Texas a condition of roses resembling mosaic. That this was a type of the disorder distinct from lime chlorosis is evident since he also reports the latter type of chlorophyll deficiency. Clinton,¹ in 1923, reported a disease of roses, resembling mosaic, on the Marchioness of Dufferin variety growing in Connecticut.

Ramsbottom (8) describes a chlorosis of rose due to excess lime in the soil. This type of chlorosis was corrected by adding 1 ounce of sulphate of iron crystals to the soil about each plant. Spraying with an iron sulphate solution, 1 oz. to 6 gal. of water, often turned the leaves green. He also stated that the stems showed yellow patches when suffering from this type of chlorosis.

The first description of rose mosaic, originally called an infectious chlorosis (10), listed three Hybrid Tea varieties as being naturally infected, and successful experiments were reported in transferring the disease to healthy plants of a fourth Hybrid Tea variety by means of buds. In the

- ¹ Letter dated May 12, 1928, to the writer from Dr. R. J. Haskell, formerly in charge of Plant Disease Survey, U. S. Department of Agriculture, Washington, D. C.
- ² Taubenhaus, J. J. (Rose mosaic). U. S. Dept. Agr., Bur. Plant Indus., Plant Dis. Rptr. Suppl. 29: 447. 1923.
- 3 ______. (Rose mosaic). U. S. Dept. Agr., Bur. Plant Indus., Plant Dis. Rptr. Suppl. 42: 361. 1925.

following year this trouble was reported to be widely distributed in green-houses of the Eastern and Midwestern States and Eastern Canada on many varieties of Hybrid Tea roses and on domestic Rosa manetti stock (11). It was further reported that the disease had been transferred by means of buds to R. multiflora and R. odorata but that attempts to transfer it to healthy roses by means of insects failed.

Nelson (7) reported this disease on Pernetiana and Hybrid Pernetiana roses in Michigan and heavy losses on Hybrid Tea varieties. He further reported that, as a result of a survey in California, from 10 per cent to 100 per cent infection was found on plantings of Rosa manetti and Ragged Robin understocks. He obtained successful transfer by means of buds and grafts but negative results with insects. White (12) reports it on 25 varieties of Hybrid Tea roses from 8 States and Eastern Canada. He was unsuccessful in transferring the disease to R. odorata and to Pernetiana and Polyantha types of roses in limited trials, although he had previously reported successful transfer to R. odorata. The apparent recovery of R. odorata plants to which the disease had previously been transferred led to some doubt as to the success of the transfer. However, in the light of subsequent experience, this apparent recovery is not unusual.

White (13) gives an extended account of the disease and reports it from 12 States and the Province of Quebec on 27 varieties of Hybrid Tea roses and 2 rose species. In a later article (14) control of the disease is suggested possible by thoroughly roguing the fields of diseased understock, combined with rigid inspection of mother plants from which buds or scions are to be taken. Two cases are mentioned in which it is indicated that some imported Rosa manetti plants were infected.

Milbrath (4, 5, 6) reports that surveys in California of roses in green-houses, fields, and nurseries failed to show any evidence of this disease on Rosa manetti and Ragged Robin roses, extensively grown and used as understocks, although it was found on several varieties. His illustrations clearly show that it was identical to the trouble in eastern greenhouses. A quite distinct type of chlorosis, called "albication," was noted on the variety Souvenir de Cladius Pernet. His survey in eastern greenhouses showed the disease to be very prevalent, and he suggests that it might be attributed to a genetic weakness of varieties that have arisen through breeding or as sports from Ophelia. He makes this suggestion in spite of successful transmission experiments (7, 10, 11, 12) and the finding of the trouble on varieties and species of rose with no genetic relationship whatever to Ophelia. It is still more difficult to understand Milbrath's statements in light of a survey conducted by Weiss and McWhorter* on the Pacific Coast, in-

⁴ Weiss, F., and F. P. McWhorter. Pacific coast survey for rose mosaic. U. S. Dept. Agr., Bur. Plant Indus., Plant Dis. Rptr. 14: 203-205. 1930.

cluding California. This survey reports fields of $R.\ odorata$ in which at least 5 per cent of the plants showed marked and unmistakable symptoms. In addition, several varieties of Hybrid Perpetual roses, as well as 1 Hybrid Wichuriana, were reported infected. No evidences of the disease were found on Ragged Robin stocks in California, contrary to the findings of Nelson, in 1929. Weiss and McWhorter state that it is conceivable that the symptoms were masked at the time of their survey. Rosa multiflora, in California, was found to show appreciable percentages of this disease in some fields. Little mention is made of their findings on $R.\ manetti$, in spite of their expressed purpose of determining the distribution of the disease on rose understocks being grown on the Pacific Coast, and in spite of its primary importance as an understock for greenhouse forcing of roses. The disease was not found on stocks of Texas Wax rose in Texas.

McWhorter⁵ describes 2 cases in Oregon, 1 of which indicates that *Rosa* manetti plants became infected naturally in the field from certain possible wild hosts. The other case described indicated that 1 bundle of imported English manetti cuttings were 100 per cent diseased. Nineteen other bundles, presumably from the same source, were 100 per cent healthy. Such a case leads to some doubt as to the homogeneity of the 20 bundles.

From this survey of the literature it is evident that the trouble known as infectious chlorosis or mosaic of the rose is widely distributed in the United States, being present in fields of understock on the Pacific Coast, as well as in eastern greenhouses, occurs on many varieties of Hybrid Tea, Hybrid Perpetual, Pernetiana, Hybrid Pernetiana, and Hybrid Wichuriana roses, as well as on the species Rosa manetti, R. multiflora, and R. odorata, and the variety Gloire des Rosomanes, or Ragged Robin, all used as understock.

During July, 1931, a rather extensive survey of rose stock and rose fields was made in France, Holland, and England. The only cases of a trouble of roses similar to mosaic as it occurs in the United States were found on certain varieties of climbing roses at Kew Gardens, London, England. (Fig. 5, B, C, D.) However, the outstanding symptom on these plants was a veinal type of chlorosis. It was accompanied in some cases with a slight speckling of the foliage. No evidences of similar symptoms were found in any fields of Rosa manetti nor on any varieties of roses in any of the 3 countries visited, which, combined, supply the trade with the greater bulk of importations of this item. This survey does not prove that the disease does not exist in these countries, for the symptoms might well have been masked at the time the survey was made.

⁵ McWhorter, F. P. Further report on rose mosaic in Oregon. U. S. Dept. Agr., Bur. Plant Indus., Plant Dis. Rptr. 15: 1-3. 1931.

DESCRIPTION OF CHLOROSES

Mosaic. The symptoms of mosaic as originally described on Hybrid Tea roses (10) and later amplified are as follows: The plants are dwarfed, the degree of dwarfing depending upon the variety, severity of infection, and environmental conditions. The dwarfing is expressed in all parts of the plant, including the roots. Buds are often imperfect, on short stems, and bleached. On seriously infected Madame Butterfly plants the blooms, instead of showing the normal light pink petals, tinted with gold at the base, are almost white. The leaves are variously distorted, with the midrib frequently bent and twisted (Fig. 1, B). The leaflets show distinct chlorotic areas, especially along the midrib, which cause the leaflets to pucker and ruffle (Fig. 1, B). The clearing of the veins is frequently pronounced (Fig. 1, A). Usually all leaflets on a leaf show symptoms, but one frequently finds one leaflet of normal size and without symptoms, while the rest of the leaflets show pronounced symptoms. When the chlorosis is confined to one side of a leaflet, distortion occurs, due to unequal growth of the two halves.

This description was based largely upon the appearance of plants of Madame Butterfly variety and has been illustrated in several articles on the subject (10, 13, 14). By experimentally transferring the disease to several rose species a variety of symptoms, previously suspected to be mosaic, have been proved to be expressions of this disease.

Milbrath (4, 5, 6) pointed out the wide variation in symptoms he had observed on different varieties. They ranged from minute speckling and occasional distortion to conditions approximating the above-described symptoms. Weiss and McWhorter⁶ also point out that the above symptoms do not always hold on types of roses grown as understock nor on some Hybrid Perpetual roses. They describe the symptoms on Rosa manetti as numerous, minute, chlorotic areas, distributed over the entire leaflet or concentrated toward the tip or periphery of the leaflet, with some of the chlorotic specks apparently interveinal.

Two distinct types of symptoms have been observed on Rosa manetti. One corresponds to that described for this species by Weiss and McWhorter (Fig. 1, D), except that there is little tendency for the chlorotic areas to be localized in any one portion of the leaflet. The other type of symptom is a more general chlorosis, giving a typical mosaic-like mottled appearance similar to cases of mild mosaic of potato with a slightly more pronounced pattern when viewed by transmitted light (Fig. 1, E). The first type of symptom has been observed on field-grown plants, while the latter type occurred on greenhouse-grown plants. Gradations between these two types of symptoms have been observed.

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⁶ Loc. cit.

⁷ Loc. cit.

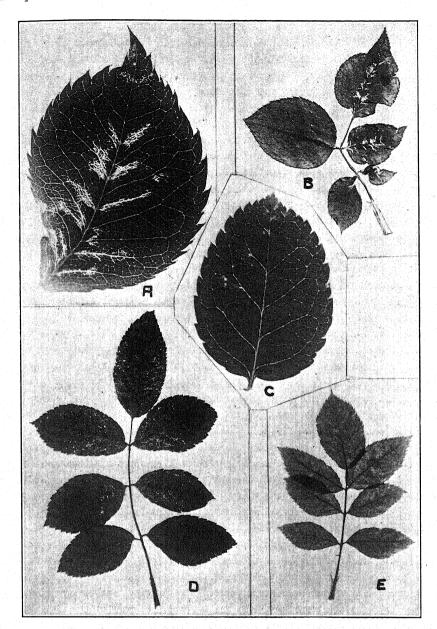


Fig. 1. A. Leaflet of Madame Butterfly variety ×4 showing clearing of veins. Natural infection. B. Leaf of Premier variety showing distortion of midrib and typical mosaic symptoms on leaflets. One leaflet apparently normal. Natural infection. C. Leaflet of Gloire des Rosomanes variety ×2 showing speckling of foliage. Experimentally produced. D, E. Two types of symptoms on Rosa manetti. Natural infections.

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The disease has not been observed by the writer on naturally infected plants of Rosa odorata. It has been transferred to this species under greenhouse conditions by using buds of the variety Madame Butterfly, taken from the bases of leaves showing typical symptoms for this variety, as described above. These buds were kept in a dormant condition. There was apparently no dwarfing of the R. odorata canes, and the symptoms, although typical and unmistakable, were slight. These plants were then cut back to within 6 or 8 in. of the crown, which forced the dormant buds of Madame Butterfly into growth. The shoots resulting from these Butterfly buds were all diseased and showed symptoms typical of this variety. The plants of R. odorata that became diseased reacted somewhat differently. Strong. vigorous shoots developed, the lower 2 feet of which showed a very marked dwarfing and distortion of the leaves and leaflets (Fig. 2, A) with typical symptoms showing upon them (Fig. 2, D). The canes in this region were also markedly mottled. A similar dwarfing of the foliage has been produced by heavy aphid infestation, but the dwarfed leaflets in such cases never showed chlorotic areas. With further growth these symptoms failed to appear and the leaves were of normal size. A third period of growth then ensued in which the leaf symptoms were again evident, although the leaves were not dwarfed.

On the older leaves of these plants another type of symptom appeared (Fig. 2, G), previously noted on naturally infected plants of Madame Butterfly and Scott's Red Premier (Fig. 4, E) and thought by some to be due to cyanide injury. These symptoms are most evident on the upper surface of the leaflets, as an irregular band of yellowish green between the midrib and the periphery of the leaflet. Frequently, the irregular leaf pattern is lost and the leaf will be blotched in definite areas, sometimes near the periphery, sometimes near the base. These symptoms are not accompanied by dwarfing or malformation of the leaflets, and appear only on the older leaves, which have reached maturity. The symptoms illustrated in figure 2, G, on Rosa odorata were produced on plants that had never been subjected to cyanide fumigation.

On Rosa multiflora a condition similar to that on R. odorata occurred. As long as the diseased buds of Madame Butterfly remained dormant the symptoms on the multiflora plant were slight. When these plants were cut back and the diseased buds were forced into activity, and strong shoots of the multiflora stock were forced to break from the bases of the older canes, the symptoms became more pronounced. In all cases the diseased Butterfly buds produced diseased shoots showing symptoms typical of the variety. The lower portions of the multiflora canes showed decidedly dwarfed and malformed leaves but no other leaf symptoms. The canes in these areas were also mottled but not so strongly as the odorata canes. Subsequent

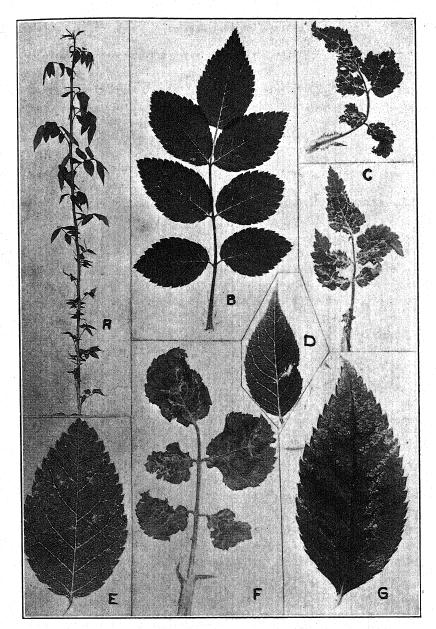


Fig. 2. A. Cane of Rosa odorata $\times 1/12$ showing dwarfing and malformation of lower leaves. Experimentally produced. B. Leaf of R. multiflora showing speckling. Experimentally produced. C. Two leaves of Texas Wax rose showing pronounced symptoms of mosaic. Natural infections. D. Leaf of R. odorata $\times 2$ showing typical symptoms. Experimentally produced. E. Leaflet of R. multiflora $\times 2$ showing speckling. Experimentally produced. F. Leaf of American Beauty budded on Texas Wax, showing mosaic. G. Leaflet of R. odorata $\times 2$ showing symptoms of mosaic experimentally produced.

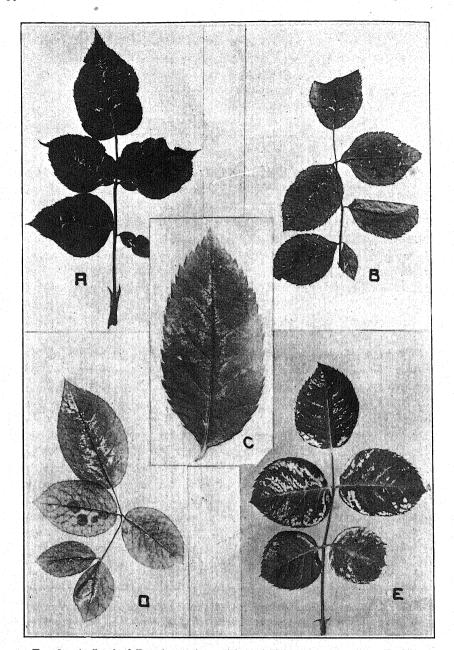


Fig. 3. A. Leaf of Premier variety with typical mosaic symptoms. B. Symptoms of mosaic on leaf of La France variety. C. Leaflet of $Rosa\ odorata \times 2$ with symptoms of mosaic experimentally produced. D. Symptoms of mosaic on Duchess of Wellington variety. E. Albication of Golden Ophelia variety.

growth was apparently normal, but there then ensued a period, corresponding to that of the *odorata* stock, in which the leaves showed a decided speckling (Fig. 2, B, E). Previous experiments on the thorny variety of multiflora produced symptoms of a more mottled type, midway between the speckling and the symptoms described for Madame Butterfly. Both types of multiflora are evidently susceptible to this disease. No evident decrease in vigor of the R. multiflora stock was noticeable in either case, under greenhouse conditions.

On the Gloire de Rosomanes variety, or Ragged Robin, widely used as understock, only the speckling type of symptom appeared. The leaves were not malformed in any way, vigor was not impaired, and dwarfing did

not result (Fig. 1, C).

The symptoms observed on Texas Wax rose,⁸ extensively used as an understock in Texas, occurred on naturally infected plants obtained from Texas and grown under greenhouse conditions. The leaves were decidedly dwarfed, distorted, and malformed. The leaflets also were extremely dwarfed and puckered and showed a very marked chlorosis, limited largely to the veins (Fig. 2, C). Some speckling of the foliage also was present, in the interveinal areas. American Beauty roses, budded on infected Texas

Wax plants, showed similar symptoms (Fig. 2, F).

That the symptoms of this disease vary with the variety has been indicated by Weiss and McWhorter. Milbrath (4, 5, 6) mentions a trouble on two Souvenir de Cladius Pernet plants that he termed albication, in which the leaves developed white patches or streaks. Figure 3, A to E, illustrates a transition from symptoms generally recognized as being associated with mosaic to those that Milbrath has termed albication. The writer has observed symptoms as expressed by both Premier (Fig. 3, A) and Golden Ophelia (Fig. 3, E) on the same plant. He also has reproduced the symptoms as expressed on the Duchess of Wellington variety (Fig. 3, D) by using buds from Madame Butterfly plants showing typical symptoms of mosaic, as described for this variety. Attempts to transfer the condition of Golden Ophelia (Fig. 3, E) to healthy plants failed because the buds used failed to unite.

NUTRITIONAL CHLOROSES

Chloroses of foliage due to deficiencies or excesses of certain soil constituents are not uncommon and have been attributed either to a lack of available magnesium, manganese, nitrogen, and iron or to excesses of nitrogen, sulphur, manganese, and calcium. Excesses of calcium increase the alkalinity of the soil to a point at which the solubility and thus the avail-

⁸ Rosa multiflora \times R. Chinensis.

⁹ Loc. cit.

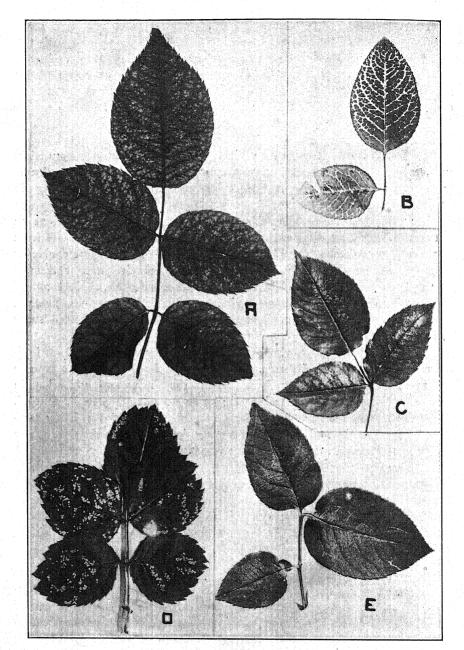


Fig. 4. A. Iron chlorosis on Premier variety. B. Veinal chlorosis on Premier variety. C. Red-spider injury on Scott's Red Premier variety. D. Leaf-hopper injury on Madame Butterfly variety. E. Symptoms of mosaic on Scott's Red Premier variety. Natural infection.

ability of certain elements essential to chlorophyll production, are greatly diminished. Chloroses due to such unbalanced soil conditions, with the exception of nitrogen deficiencies, are expressed as interveinal chloroses, and, as the condition becomes severe, the entire interveinal area becomes devoid of chlorophyll and yellow. The last places in the leaves to lose chlorophyll are about the larger veins. Deficiencies of nitrogen are expressed as a uniform yellowing of the entire leaf.

Lime chlorosis is not uncommon on roses grown in eastern greenhouses, appearing more prevalent and serious in the spring than at any other time of the year. It is frequently due to excess alkalinity of the soils in which the plants are growing. That it may also be caused by other factors is evident, for roses growing in soil with an acidity of pH 5.5 to 6.0 have been seen showing interveinal chlorosis, commonly called iron or lime chlorosis

(Fig. 4, A).

Such types of chloroses cannot be mistaken for mosaic. The light yellow-green areas occur regularly scattered over the entire leaflet, are at first faint, but become increasingly more pronounced, and are not accompanied by any type of leaf malformation. In severe cases, the new growth may appear yellow and older leaves may fall. The variety Souvenir de Cladius Pernet is especially subject to this chlorosis when grown under glass. The condition is correctable. Ramsbottom (8) reports that iron sulphate, worked into the soil or spraying the plants with iron sulphate solutions has brought about recovery. The writer's experience has been that the use of soil-acidifying materials will bring about recovery in those cases that are due to excess alkalinity of the soil. Such methods have no effect on the elimination of the symptoms of mosaic.

This condition also is not to be confused with etiolation. Milbrath (4, 5, 6) states that cases of mosaic are difficult to separate from cases of etiolation. Etiolation is a term denoting loss of chlorophyll in leaves combined with an increase in the distance between nodes. It is brought about by growing plants in darkness and can be mildly simulated by decreased light intensities. There is no lengthening of the nodes of rose plants infected with mosaic; in fact, the reverse is often true. The loss of chlorophyll in etiolated plants is uniform over the entire leaflet, contrary to the manner of chlorophyll loss in leaves of mosaic-infected plants, which occurs in isolated areas surrounded by tissue of normal color. Furthermore, mosaic-infected plants exhibit chlorosis in full sunlight, which would not result if the symptoms described for mosaic were due to light deficiencies.

VEINAL CHLOROSIS

Occasionally there is found a plant some of whose leaves show a marked veinal chlorosis (Fig. 4, B). The only cases observed were in a house of

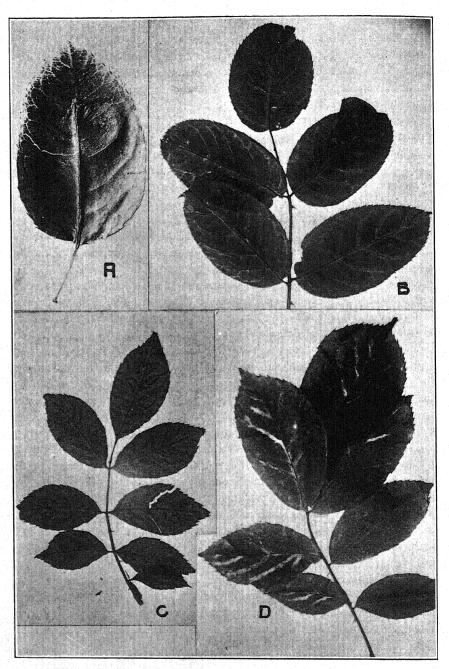


Fig. 5. A. Cyanide injury on Talisman variety. B. Veinal chlorosis on Multiflora variety Helene. C. Veinal chlorosis on Hybrid Wichuriana variety Sander's White. D. Veinal chlorosis on Multiflora variety Ariel.

Premier variety, which showed 35 per cent of the plants with typical mosaic symptoms, and a few roses in Kew Gardens, London, England. The few plants observed showed only the veinal type of chlorosis, and its relation, if any, to mosaic is not known. The leaves were not distorted in any way and the plants were not visibly dwarfed. Such distinct limitations of the chlorosis to the veins has not been observed in any of the plants to which mosaic has been experimentally transferred. The similarity of symptoms, however, as expressed by the variety Ariel (Fig. 5, D), to those illustrated by Rawlins and Horne (9) for a graft-infectious disease of the cherry is striking, with the exception of the color reaction expressed by the two hosts.

INSECT CHLOROSES

It has been the opinion of some that the condition referred to as an infectious chlorosis or mosaic was but the result of direct insect injury, 10 and many investigators, unfamiliar with the disease, are inclined to this view.

Milbrath (6, 7, 8) reports that some of the symptoms he observed in a survey of eastern greenhouses bore a strong similarity to injuries caused by various agencies, such as thrips, red spiders, and aphids, all of which, he said, were primary pests on roses. There are only two such pests on roses in eastern greenhouses, one a mite (red spider) and the other an insect (aphid). Thrips, in eastern greenhouses devoted to rose forcing, are not a primary pest, and attempts to colonize them on roses failed under winter greenhouse conditions. The rose midge has been present in isolated houses but is not commonly encountered. The rose midge injures by chewing. It feeds upon the young growing tips and undeveloped buds and causes malformed leaves, injured blooms, and a stunted condition or death of the terminal growth. No chlorosis of the foliage is produced and mosaic, therefore, cannot be attributed to the presence of this pest.

Leafhoppers are not common on roses under glass, and attempts to colonize them on greenhouse roses during the winter months have met with little success. Their presence results in a loss of chlorophyll at the point of feeding, causing a type of injury so distinct from any of the symptoms produced by mosaic that the two cannot be confused (Fig. 4, D). Furthermore, mosaic may occur in the absence of leafhoppers and it is therefore evident that this insect pest cannot be the direct cause of this disease.

Red spiders are very common on greenhouse roses. They colonize on the under surface of the leaflets, causing injuries very similar to those produced by the leafhopper, except that the individual feeding points are much smaller. On the upper surface of the leaves, above these colonies,

10 Johnson, K. M. S. Conference on importation of fruit and rose stocks. Fed. Hort. Board. Mimeog. Rpt., p. 51, June 27, 1928. Washington, D. C.

mottled greenish gray areas appear (Fig. 4, C). The chlorosis produced is quite dissimilar to the yellowish or yellowish green produced by mosaic. Furthermore, the spider, in various stages, from the egg to the adult, can be found on the under surface of spider-injured leaves. Mosaic symptoms are not correlated in any way with colonies of red spider and have appeared on plants on which no spider has ever been present.

Aphids (the common green fly of the commercial grower) are also common on greenhouse roses, clustering about the growing tip and sucking out plant juices, causing a stunting of growth and, if abundant, distortion of foliage. Aphids, feeding on young foliage before it unfolds, frequently produce on that foliage a scattered yellow speckling. This might be mistaken for mosaic, if no other symptom of the disease are present, especially if the speckling is observed after the aphids have migrated to other plants. It usually is not difficult, however, to determine whether the speckling is due to aphids or to mosaic. Aphids never cause the pronounced symptoms typical of mosaic. Furthermore, they have never been seen in sufficient numbers to account for the effects of mosaic in commercial rose houses where periodic fumigation is practiced and where mosaic is general.

In the fall of 1929, 6 mosaic-infected plants of Madame Butterfly were brought from the field to the greenhouse, cut back severely, entirely stripped of leaves, and planted in a ground bed in insect-proof cages. Normal growth took place for a period of 8 weeks, at the end of which time a few typical symptoms of mosaic appeared on 1 of the plants. At the end of 11 weeks all 6 plants were showing typical symptoms of mosaic, without the association of an insect of any kind. It is evident that the symptoms expressed by this trouble are not due to direct insect association.

CYANIDE INJURY

Fumigation with cyanide for the control of aphids is a common practice in eastern greenhouses. Excess dosage will produce a chlorosis of rose foliage but a type of chlorosis quite distinct from that produced by mosaic. With cyanide injury the chlorophyll is destroyed around the margins of the leaflets and is more severe and extensive at the tip. The chlorosis extends down the veins (Fig. 5, A) and is a bright yellow to a bleached whitish color. In severe cases of cyanide injury the injured tissue becomes brown and dead. This chlorosis shows up shortly after fumigation and can be diagnosed correctly without difficulty.

DISCUSSION

The possibility of this disease being due to direct insect injuries is precluded by the production of typical mosaic symptoms on foliage of naturally infected plants grown in insect-proof cages, without any insect association. Furthermore, the injuries produced by rose insects are quite distinct from the symptoms of mosaic. Likewise, chlorosis produced by unfavorable soil and atmospheric conditions, and the destruction of chlorophyll by cyanide fumigation, are readily diagnosed and easily distinguished from mosaic.

Milbrath (4, 5, 6) states that the symptoms of mosaic are difficult to separate from types of chloroses due to etiolation, icterus, and albication. The chlorosis due to etiolation is initiated by light deficiencies and is of a uniform character, which will not permit it to be confused with mosaic. Similarly, conditions described as icterus, in which the chlorophyll of plants becomes deficient, are due to unfavorable growing conditions and are non-transmissible and the yellowing of leaves is uniform, in marked contrast to the speckling, mottling, and isolated chlorotic areas due to mosaic infections.

Conditions of albication, in which the leaves or portions of leaves develop whitish or yellowish areas, do approach the pronounced symptoms of mosaic. The term albication does not imply that the condition is not transmissible. In fact, the infectious chloroses with which Baur (1, 2, 3) worked were albications or variegated forms of Ligustrum, Laburnum, Malva, etc. Milbrath, himself, speaks of the albication he found on Souvenir de Cladius Pernet as a "definite instance of a transmissible condition of albication" (5, 6). However, he merely proved by his experiments that the condition was perpetuated by vegetative means. He did not obtain a transmission of the condition to the plant to which he transferred buds of the albicant.

The wide variation in symptoms known to be transmissible and associated with mosaic and the fact that symptoms of both albication and mosaic have been found on the same plants of Golden Ophelia indicate that the albication of the rose may be a pronounced symptom of mosaic. (Fig. 3, A-E). Some plants of the variety Conrad Ferdinand Meyer, growing in the New York Botanical Gardens, which showed pronounced symptoms of albication, were brought to my attention in 1929 by B. O. Dodge. In 1930 these same plants and, in addition, plants of the variety A. K. Williams, not far removed, showed the same symptoms. This same condition has been observed on plants of the Premier and Butterfly varieties growing outdoors, which had been transplanted from the greenhouse and which originally showed only typical mosaic symptoms.

The perpetuation of the symptoms of mosaic has been proved beyond any doubt by numerous budding experiments. Buds from infected plants will always produce infected shoots. Furthermore, in a considerable percentage of all trials, the entire plant, originally healthy, into which diseased buds are inserted, will become diseased. In such cases a transmission of the disease to the budded plant has taken place. These cases do not

constitute merely the perpetuation of a character by vegetative means but prove the transmissibility of the abnormal condition.

Milbrath also points out that "recovery has been very significant in this trouble of the rose" (4, 5, 6). In support of this statement he cites one case in which the disease in a period of 2 months had decreased from 50 per cent to 24 per cent. Another case was cited in which several diseased plants were cut back and started anew and at the end of 4 months were reported as having recovered. Other similar instances are given.

Following severe pruning, growth as a rule takes place normally for varying periods of time. Plants have been grown for 3 years under outdoor conditions, but they have always shown the typical symptoms of mosaic at some time during the growing season. The expression of the symptoms of this disease seems to be particularly sensitive to environmental conditions, and the failure to exhibit typical symptoms is no proof that the plants are recovered. Plants, known to be infected, have been carried from a severe pruning to bloom without any symptoms appearing. A second severe pruning, however, was again followed by new growth and this growth exhibited typical symptoms in due time. Until further studies can be made upon environmental conditions, as related to symptom expression, this reaction will remain unexplained. In no case have I ever seen recovery, meaning absolute freedom of the trouble. Plants may and normally do fail temporarily to show symptoms following heavy cutting back and in response to certain unknown environmental conditions but, eventually, with the return of proper environmental conditions, the symptoms will again be expressed.

Milbrath further states (4, 5, 6) that, if this were a virus disease, it would be systemic. Buds have been taken from canes of infected roses from bases of leaves, some of which were showing symptoms and some of which were not. In cases where the buds used formed a union and grew successful transfer of the disease was accomplished by buds from bases of leaves that were apparently normal and healthy, as well as by buds from bases of leaves showing pronounced symptoms. The failure of some leaves on infected plants to show symptoms cannot be explained by assuming that rose mosaic is nonsystemic. The absence of symptoms on certain leaflets, while other leaflets of the same leaf show pronounced symptoms, is not infrequent. Leaves developed at the same time on different shoots of the same plant or on different plants, under exactly similar external environment, may or may not show symptoms. Such observations would indicate a different internal environment. It has been noted that plants, high in carbohydrates and low in nitrogen, often fail to show symptoms when diseased buds are united with them.

SUMMARY

The symptoms of the infectious chlorosis of rose known as mosaic are given for various varieties and species of rose and compared with other types of chlorosis.

The species Rosa manetti, R. multiflora, R. odorata, and the variety Gloire de Rosomanes, or Ragged Robin, as well as the variety known as Texas Wax, all used as understocks, have been found susceptible. The symptoms of mosaic on these species and varieties may be quite distinct from the symptoms expressed by certain Hybrid Tea varieties.

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A STUDY OF LABORATORY METHODS FOR INVESTIGATING THE RELATION BETWEEN MOISTURE CONTENT OF WOOD AND FUNGAL GROWTH

FERDINAND W. HAASIS1

INTRODUCTION

General procedure. This study was conducted in the Forest Products Laboratory at the School of Forestry, University of Idaho, during the years 1928–1930. It constituted one phase of a comprehensive study of the optimal and minimal moisture contents of wood for the growth of decay-producing fungi, under the general supervision of Dr. E. E. Hubert, of the School of Forestry. The chief immediate objective was the development of a relatively simple technique whereby studies of the relation between moisture content and fungal activity might be satisfactorily made. Acknowledgment is made to Doctor Hubert for suggestions and criticisms in connection with both experimental work and presentation of the results.

In these experiments the only host used was sapwood of western yellow pine, *Pinus ponderosa* Dougl. The saprophytic organism used was *Fomes roseus* (Alb. and Schw.) Fr., which caused a brown pocket rot. This species occurs in nature on the wood of western yellow pine (see Anderson, *et al.*²), although the strain used was a pure culture isolated by Doctor Hubert, from jack pine, *Pinus divaricata* Du Mont de Cours. The cultures were incubated at a temperature of about 27° C. No study was made of the effects of varying amounts and qualities of light in the culture chamber. Incubation was continued for a period of approximately 6 months, 180 days for the most part.

In experiments of this type the activity of the fungus is considered to be represented by the relative loss in oven-dry weight of the wood. This is a method that has been used to a considerable extent in such studies (cf. Humphrey, p. 265, Schmitz, Hubert, pp. 14, 16, 17).

- ¹ Carnegie Institution of Washington. Formerly, School of Forestry, University of Idaho.
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- ⁵ Hubert, E. E. A study of laboratory methods used in testing the relative resistance of wood to decay. Idaho Univ., School of Forestry Bul. 3. 1929.

RESULTS AND DISCUSSIONS

Addition of moisture. At first sticks of wood about 14 cm. long and 1 cm. square were used. These were oven-dried at 100° C. to constant weight and then a small amount of water was added so that about 1 cm. of the lower end of the stick was immersed. It was hoped that the water absorbed by the stick would become so distributed that a moisture-content gradient would result. It was planned, such a gradient having been established, to place some inoculum near the middle of the stick and, after a period of incubation, to study the activity of the fungus at various heights.

It was soon learned, however, that, although the water would creep up the sides of the stick and even over the top, its progress through the stick was much slower. It was evident that the results of moisture determinations in samples from these sticks must be accepted somewhat cautiously because of the irregularity of the moisture distribution. This irregularity, it was found, can be very graphically demonstrated by the use of indelible pencils.

Obviously, in making studies of the relations between moisture content and decay, it is essential to have the water distributed with considerable uniformity through the test material. Accordingly, attempts were made to insure uniform distribution of added water.

In these experiments the length of the test pieces was much reduced, blocks about 2.5 cm. long and 1.3 cm. square being substituted for the sticks. These blocks were weighed individually, the desired moisture content was decided upon, and the water to be added was computed, assuming the air-dry wood to contain the same amount of moisture as check blocks whose moisture content was ascertained. This amount of water was placed in a cotton-plugged test-tube with the block and the preparation autoclaved under various conditions. Individual preparations were, for example, autoclaved at 2-lb. pressure for 45 min., cooled, and autoclaved at 5-7-lb. pressure for 30 min.; autoclaved at 5-lb. pressure for 30 min.; and autoclaved 30 min. at atmospheric pressure, and, 2 days later, for 30 min. at 7-lb. pressure.

The moisture actually absorbed under these conditions seemed to have little relation to the amount intended. Therefore, flat-bottom vials were substituted for test-tubes, and a number of different autoclaving methods were tried; but the moisture distribution, as indicated by indelible-pencil streaks, was not entirely uniform with either cotton plugs or toy-balloon caps. Of the methods tried, autoclaving for 1 hour at atmospheric pressure (98.5° C., as ascertained by a number of observations) seemed to give the best results. Petri-dish autoclaving gave unsatisfactory moisture distribution.

Acting on a suggestion of Doctor Hubert's, Sitka-spruce veneer (0.03 cm. thick) also was given a trial, 5-cm. squares being placed in individual Petri dishes and water added to each dish. It was thought that better distribution might be secured in these thin pieces. It was found, however, that the distribution was even more irregular than in the case of the blocks. It is to be borne in mind that the amount of water added was proportional to the weight in both cases, and with the veneer squares was very small, indeed (0.04–0.15 gm.). Condensation on the inside of the Petri dish was very pronounced, and this resulted in spotty moisture distribution in these small pieces. It was observed, also, that such thin sheets may be moist on one side and dry on the other (0.01 in. away.)

The idea suggested itself that, if the blocks were heated when placed in water, the absorption might be more uniform than theretofore. Accordingly, a couple of sets that had been thoroughly warmed by being kept in a 100° (C.) oven 3 days were placed in water in vials and then autoclaved an hour at atmospheric pressure. The moisture distribution in these blocks,

however, proved to be not notably uniform.

Stender dishes were used next as containers during the autoclaving. There were available dishes 4 cm. high and 8 cm. in diameter that would conveniently hold 10 of the $1.3 \times 1.3 \times 2.5$ cm. test blocks. These dishes had ground edges and ground grooves in the lids fitting over the edges. The blocks were autoclaved an hour at atmospheric pressure. This method was found eminently satisfactory and was tentatively adopted as standard, the blocks being preferably autoclaved the day after adding the water and left a day after autoclaving, before using.

One comparative test was run using the following containers:

Two blocks lying on a long side in a small stender dish (about 2 by $4 \,\mathrm{cm.}$);

Two blocks lying on a long side in individual small stender dishes;

Two blocks in cotton-plugged vials;

Two blocks in cotton-plugged test tubes;

Two blocks lying on a long side, in a deep Petri dish.

In this test the moisture distribution was fairly uniform in one block autoclaved in an individual stender dish, in one block in a vial, and in the two blocks in the Petri dish. In the others it was not very uniform.

The 10 test blocks of a series were habitually weighed individually and water enough added to the lot in the stender dish to give the desired average moisture content. The results were not very precise, the individual blocks of a lot absorbing widely different amounts, although the distribution was uniform in each block. The average moisture content of a lot could, however, be approximated fairly closely. The following tabulation, while including a considerable number of interpolated figures, will give some idea

of the amounts of water required in using this method. The figures apply to western-yellow-pine sapwood blocks of medium grain, approximately $1.3 \times 1.3 \times 2.5$ cm. in dimensions and averaging 6 per cent moisture content.

Average moisture content desired 10 blocks, per cent	Approximate amount of water to be added, ml.	
10	1.0	
20	3.0	
30	5.0	
40	7.0	
[44] [1] 4 4 1 [1] 4 4 50 [1] 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4	9.0	
60	11.0	
70	13.0	
80 min 1 min	15.0	
90	17.0	
100	18.5	
110	20.0	
120	21.5	
$_{130}$	23.0	
140	24.5	
150	26.0	

In order to ensure a close approximation to the desired absorption by individual blocks, it would probably be necessary to use a separate dish for each block. Needless to say, the more uniform in texture, specific gravity, and air-dry moisture content the test material is, the more precisely can the desired amount of water be added and the more significant will be comparisons of decay in blocks of diverse moisture content.

Maintenance of uniform moisture content. While a satisfactory method of adding moisture to the test blocks has been developed, the problem of maintaining the moisture content during an incubation period of as much as 6 months has not yet been solved.

Following a method developed by Hubert, culture tubes plugged with cotton were capped with toy balloons. In reweighing such tubes, after from 10 to 40 days in the culture chamber, it was found that they had lost weight at the rate of about 0.05 gm. a day. A study was accordingly made of the amount of water lost during a period of 27 days through a number of different stoppers, two of each type being tested. At the beginning of the experiment 20 ml. of water was added to each of 10 test tubes and the several stoppers then applied. The average losses in weight were as follows:

⁶ Loc. cit., p. 38.

	27 days	Per day	Per 10 days
Cotton stoppers	3.79 gm. 1.30	0.14 gm. 0.05	1.40 gm. 0.48
Cork stoppers (No. 12) with transverse lenticels	0.28	0.01	0.10
Paraffined cork stoppers, similar to above	0.04		0.01
Rubber stoppers (No. 5)	0.07		0.03

From these experiments it is evident that the paraffined cork was the most effective stopper tested, in preventing loss of moisture. The plain corks were the most variable, the 27-day losses being 0.44 gm. and 0.12 gm., respectively. The variation in the other pairs was relatively slight.

From this study it appeared that in using the balloon-cap method of covering test-tubes there can be expected a considerable loss of water, though this loss is only about $\frac{1}{3}$ that that occurs without the caps. Yet, this loss might quite appreciably affect the results in studies of the influence of moisture content. It seemed that for such work a tighter stopper would be much preferable; and paraffined corks were, accordingly, tentatively adopted as standard for the cultures started after May, 1929.

There also should be mentioned here a test of sodium silicate as a coating for cork stoppers. For this purpose the corks were dipped in a commercial solution of the material (water glass). This is perhaps easier to apply than hot paraffin. It has, however, certain very pronounced disadvantages. If dried on a glass plate, the treated corks stick much more than do paraffined corks, allowed to cool on a glass plate. Double dipping is advisable in using either water glass or paraffin; the paraffin sets more quickly than the sodium silicate solution. After 15 days of incubation most of the water-glassed stoppers were found to be quite firmly stuck to the tubes, a trouble not experienced with paraffined stoppers. The sodium coating is less flexible than one of paraffin, and it has a tendency to develop sharp projections on the corks, a drawback absent in the case of paraffin.

Corks coated with either sodium silicate or paraffin cannot be autoclaved without ruining the covering. It is possible, however, that water-glassed corks might be successfully sterilized in an oven. No significant difference in growth was observed between the fungus in tubes stoppered with water-glassed corks and that in tubes stoppered with paraffined corks.

Even with the use of the paraffined corks, there was some loss of moisture from the cultures, although appreciably less than from the cotton-plugged cultures. During an incubation period of about 6 months the average daily loss from the cork-stoppered tubes varied from 1.3 mgm. to 10.9 mgm. for the tubes containing the inoculated blocks and from 1.6 mgm. to 10.3 mgm.

for the controls. Corresponding figures for the cotton-plugged tubes are 16.8 to 48.6 mgm. and 15.5 to 42.9 mgm.

The tubes that were plugged with cotton contained agar gel about 4 or 5 cm. deep, whereas most of those with the cork stoppers had considerably less agar or practically none at all. It might be thought that perhaps the greater losses could be attributed to the fact that there was more moisture in the tubes. However, two tubes of a set with deep agar were stoppered with corks instead of cotton (with rubber covering) with the following results:

	Average daily losses	180-day in- cubation
	Inoculated	Control
Cotton-and-rubber-stoppered tubes (average of 4) Cork-stoppered tubes	16.8 mgm. 1.4	15.5 mgm. 2.6

From these figures it is evident that the lesser losses from the cork-stoppered tubes are at least not entirely due to their smaller water content.

TABLE 1.—Average daily loss in weight from cultures during an incubation period of about 6 months

Original average moisture content of blocks, per cent (based on oven-dry weight)	Number of blocks averaged	Amount of loss, mgm.	
		Inoculated blocks	Contro blocks
21	8	1.3	
24	2		1.6
33	2		2.7
35	8	2.8	
35	2		3.5
38	2		3.6
40	2		3.4
46	8	3.7	
51	8	3.7	
53	2		3.1
60	8	5.6	
65	2		5.7
78	8	4.9	
80	8	4.8	
108	5	5.1	
113 de la 113 de	5	5.5	
113	5		6.1
120	5		5.4
141	8	6.8	
143	2		10.3

That there is, however, an approximate relation between the water content of the tube and the losses in weight is indicated by the figures of Table 1 obtained from a number of series of cultures prepared in a very uniform way, with only a small amount of agar in the tube, a piece about 0.5 cm. square placed upon the test block.

If figures for individual cultures be used, the irregularities are naturally found to be somewhat greater than in the averages just presented. For example, the loss from one inoculated culture, in which the original moisture content of the block was 107 per cent, was 10.1 mgm. per day, while, in another, with original moisture content of 111 per cent, the corresponding loss was only 2.9 mgm. For the control blocks of the series under consideration the figures are given in Table 2.

TABLE 2.—Average daily loss in weight from individual control cultures during an incubation period of about 6 months

Original moisture content of blocks, per cent	Amount of loss, mgm.	Original moisture content of blocks, per cent	Amount of loss, mgm.
24	1.6	58	5.4
24	1.6	60	1.4
28	2.3	72	5.9
34	3.4	105	7.9
35	3.7	114	8.7
36	3.1	116	8.5
36	3.8	118	1.7
38	3.1	124	4.2
40	3.3	137	4.8
43	3.6	171	12.0
45	4.8	•	

It will be noted that, in spite of certain unexplained irregularities, the general trend of the figures indicates a rather direct relation between the amount of weight lost during incubation and the water in the preparation available for evaporation.

A comparison was made of the amount of weight lost by the tubes as a whole and by the blocks of the control preparations. Considering, first, the tubes containing a considerable amount of agar gel (about 20 ml.), it was found that the loss from the blocks was appreciably less than from the preparation as a whole. Evidently, there was a considerable loss of moisture from the agar gel. In a few instances the blocks gained in wet weight during the test period. This is undoubtedly due to an absorption from the agar, faulty technique allowing the blocks to come into contact with

the gel $(cf. \text{ Hubert}^{7})$. These tubes were stoppered with cotton and balloons.

In a number of series, the agar in the tube was considerably less than in those just discussed, varying from 0.5 ml. to 2 ml., mostly 1 ml. These tubes were stoppered with paraffined corks (or in one series with water-glassed corks). In some of these series the water loss from the blocks was greater than the loss from the preparation as a whole. This can readily be explained by assuming that the agar gel absorbed moisture that had vaporized from the block.

There are now to be considered the series in which almost no agar was used, the tubes being stoppered with paraffined corks. As in the case of the tubes containing large quantities of agar, there was, for the most part, a somewhat greater loss from the preparation as a whole than from the blocks, the difference averaging about 0.4 mgm. a day. Apparently there was some loss of weight from the stopper. It is possible that bits of solid paraffin, to the amount of 72 mgm., were lost from the preparation during the 6-month period, though it seems very unlikely. It is just as unlikely that such a loss is attributable to vaporization of moisture from the interior of the cork stopper at the temperature used or to the volatilization of constituents of the thin coating of paraffin used.

In these preparations containing a minimum of agar there seems to be a tendency, when the original moisture content of the block is more than about 117 per cent, for the block to lose more weight than the preparation as a whole. Since it is very unlikely that any appreciable amount of other materials than water is lost from the block during a 6-month period, this loss from the blocks must result in an increased humidity of the air within the tube.

In some tubes, containing 2 ml. of agar, or only a small amount, it was observed that dew had formed on the walls, presumably the result of condensation, when the tubes were taken out of the relatively warm culture chamber for inspection. Sometimes there was even free water in the bottom of the tube, 1–6 mm. deep. In a few cases free water was noted on top of 2 ml. of agar, below the mycelial mat when present. Such circulation of the water within the tube undoubtedly results in a slightly irregular distribution of moisture within the block, with a tendency for the outside to be a trifle moister than the inside. These differences, however, would be very difficult to demonstrate.

It seems that if paraffined stoppers are to be used in such work, special precautions must be taken to keep them firmly fixed in the mouth of the culture tube. It was noted time and time again, during routine monthly

⁷ Loc. cit., p. 34.

examination of the preparations, that some of the stoppers had become loose, rising above their former seating.

In a few cases, an appreciable gain (6–11 per cent) was noted in the oven-dry weight of the test blocks. Presumably, this is due to the absorption of solid substances from the agar gel by blocks in contact with it (cf. Hubert).⁸

Oxygen supply. The use of a relatively tight stopper introduces the problem of exhaustion of the oxygen in the culture tube with the subsequent slowing down of the activities of the fungus. One method suggested, by Hubert, of overcoming this difficulty is to replenish the oxygen supply at intervals during the incubation period. Perhaps the simplest method of effecting this would be to open the tubes under aseptic conditions and decant the accumulated carbon dioxid, allowing a new supply of air to flow into the tube.

In view of the large capacity of the tubes used (78 ml. with stoppers in place) and the relatively small size of the test blocks (about 4 cm. 10) it is possible that during a 6-month incubation period the oxygen exhaustion would not prove serious. This could be easily tested by preparing sets of similar cultures and decanting the carbon dioxid from some of the tubes at intervals, leaving some cultures till the end of the incubation period without such treatment; but this was not done in the studies under discussion.

When carbon dioxid is formed by the respiration of the fungus in a testtube, it will, as is well known, settle to the bottom, and diffusion of the oxygen into this lower layer will be exceedingly slow. It is conceivable, therefore, that fungal growth might be inhibited at the bottom of a testtube while there was still considerable oxygen in the upper part of the tube. It seems certain that such a result could be postponed by elevating the test block above the bottom of the tube, as on a short section of thin glass tubing. One set of tests with this method, however, yielded inconclusive results.

It might be thought that an absorbent of carbon dioxid, such as soda lime or potassium hydroxid, could be used in the bottom of a culture tube to take up the carbon dioxid as formed, allowing the remaining oxygen to reach the growing fungus. Unfortunately, these substances absorb water as well as carbon dioxid, and test blocks placed in tubes containing them lose moisture to a very pronounced extent.

The following specific figures will make this clearer. In 5 sets of 5 non-inoculated blocks, each, varying in average moisture content per block from 110 per cent to 174 per cent, and placed in tubes containing soda lime,

⁸ Loc. cit., p. 34.

⁹ Loc. cit., p. 38.

¹⁰ Humphrey, loc. cit.

the average moisture content at the end of 6 months ranged from 11 per cent to 26 per cent. In these tests the average daily loss in weight from the blocks was considerably in excess of that from the tubes, ranging from 2 to 13 mgm. more. However, from each of the 5-tube sets there was a net loss amounting to an average per tube of 3 to 9 mgm. a day. While the available figures are not numerous enough to show the fact definitely, there is an indication that this net loss is very close to that from the tubes with almost no agar and, presumably, is attributable to the same causes, whatever they may be. At any rate, it seems evident that under the conditions of these tests, the soda lime does not absorb an appreciable amount of carbon dioxid or water from the air surrounding the tubes.

In these tests the soda lime was placed in short lengths of glass tubing, about 8 cm. long, plugged at each end with cotton (or of glass wool or asbestos fibers) and then, having been placed in the test-tubes, sterilized and dried in an oven. After as short an incubation period as 30 days it was noted that the soda lime was deliquescing and saturating the lower cotton plug. At the end of 2 months there was free liquid in the bottom of some of these culture tubes to a depth of 0.5 to 1.0 cm. These effects were less marked in the case of glass-wool plugs, and still less so in the case of asbestos. There appeared to be less dew on the test-tube walls in the presence of soda lime than in comparable preparations without the soda lime.

Inoculation methods. Several different methods of inoculating the test blocks were tested. In the earliest work, the fungus was established, during a period of a week or 2, on about 20 ml. of 1.5 per cent nutrient agar gel in the bottom of a test-tube. Small sterile glass plates, about 1.3 cm. square and a couple of millimeters thick, were then placed upon the gel and the test blocks introduced on top of these. This method resulted in good fungal growth on the blocks. In the case of the control blocks, however, there was a tendency for the plates to sink down into the gel, allowing the blocks to absorb moisture from the agar. It may be that the inoculated blocks did not so absorb moisture because of the support offered by the fungal mat on the surface of the agar. On the other hand, it seems possible that water is brought up through the fungal hyphae themselves, when they are in contact in this way with a moist mass of agar gel.

Two glass plates as supports proved no more satisfactory than one in preventing increase of moisture content. When the glass plates were supported on a glass tube driven down into the agar gel, however, the increase in moisture content did not occur. The use of more concentrated agar (3 per cent) with glass plates on top also eliminated moisture absorption by the blocks.

Still the question remained as to whether a smaller quantity of agar gel might not be preferable, and several sets of cultures were prepared with 2

ml., 1 ml., or 0.5 ml. of agar gel. A test of a couple of air-dry blocks placed directly upon 1 ml. of 1.5 per cent agar indicated that there would be inappreciable absorption of moisture by blocks under such conditions. As it turned out, there was a consistent loss of moisture, during the 6-month incubation period, from the blocks in the tubes with small amounts of agar.

Another method of inoculating involved the use of a small bit of mycelium, about 0.5 cm. square and perhaps half a mm. thick, placed directly upon the block in the bottom of the test-tube, or sometimes upon the wall of the test-tube, adjacent to the block. The conrols for such test blocks were provided with bits of nutrient agar of approximately the same dimensions.

Infection of the test blocks resulted from all methods of inoculation, although not in all instances. In a few cases abortive sporophores were produced within 6 months, both when 1 or 2 ml. of agar gel were placed in the tube and when a small amount of inoculum was used.

When the inoculum is placed upon the tube wall rather than upon the block, it seems desirable to leave the preparation lying on its side for about 2 days, with the block in contact with the infected agar gel.

Sterilization. The agar solution was sterilized in the autoclave, at relatively low pressures, in accordance with customary laboratory technique, cotton plugs being used for the test-tubes. Glass plates, glass tubes, and soda lime were usually baked for a number of days in a 100° C. oven. Occasionally, the plates were dipped in ethyl alcohol, which was then burned off. When blocks were to be placed in empty test tubes, these, too, with cotton plug, were oven-sterilized.

Sterilization of the blocks was considered effected during the process of adding water by autoclaving at 98.5° C. That this treatment probably had the desired result is indicated by the facts that no wood-destroying fungi were noted on the control blocks and that significant losses in oven-dry weight were not observed for the control blocks. It is not certain that the method would be applicable to the study of resinous heartwoods.

The most troublesome problem of sterilization concerned the stoppers. As Hubert¹¹ has mentioned, mold is apt to develop in capped cotton plugs. If the upper walls of the tube bear the slightest trace of nutrient agar, these molds are very likely to creep down the walls and cause contamination of the agar at the bottom and of the test block.

It was thought that the use of paraffined corks might obviate this difficulty. The paraffin was not hot enough to sterilize the corks, but they were habitually dipped in ethyl alcohol and allowed to dry before substituting them for the cotton plugs used while sterilizing the culture tubes. In spite of this treatment, molds of unidentified genera, but probably species of Penicillium, Rhizopus, and Aspergillus, developed upon the por-

¹¹ Loc. cit., p. 38.

tion of the stopper within the tube in a great many cases and spread to the agar and to the blocks even in preparations where there was almost no agar in the tube. One set of tubes stoppered with freshly paraffined corks not dipped in alcohol seemed no worse contaminated than when the alcohol bath was used.

It is possible that contamination of the stoppers took place while the alcohol was drying from them. In view of results from exposures of nutrient agar under somewhat similar circumstances, however, this explanation seems somewhat untenable. Another possible explanation is that the mold spores were on and perhaps some distance inside the stoppers, that they were protected from the alcohol by the paraffin coating, and that the mycelium eventually grew through the paraffin coating. It is desirable to try oven-sterilizing of the corks just prior to the paraffin treatment.

MISCELLANEOUS DETAILS OF TECHNIQUE

In the manipulations involved in these studies, a number of practices and conventions were adopted and are here recorded as suggestions for future workers on similar problems.

When cotton plugs were replaced by paraffined corks, any adhering bits of cotton were habitually removed from the throat of the tube by means of sterile forceps.

In making the preparations, the blocks were weighed in the air-dry condition and a computed amount of water added on the basis of the summed weights. The air-dry moisture content of the blocks was approximated by ascertaining the moisture content of similar blocks from time to time. The culture tubes were weighed with agar, glass plates, etc., and cotton or cork stoppers just before adding the blocks and again immediately afterwards. The difference in weight gave the wet weight of the block. The moisture content was then computed on the basis of this weight, the air-dry weight, and the air-dry moisture content. When rubber caps were used, the tubes were weighed a third time after the addition of these.

Gross-weight weighings of the preparations were made at intervals during the incubation period, usually not oftener than once a month. At the completion of incubation the fungus was removed from the outside of the test blocks and the block weight ascertained.

SIGNIFICANCE OF MOISTURE CONTENT

On the basis of the study as conducted to date, no attempt at a statistical treatment of the results from the standpoint of favorable moisture content would be profitable because of the failure to develop a technique for maintaining the moisture content reasonably uniform. It is impossible, at the present stage of this study, to make any generalized statement about optimal or minimal moisture content. Inasmuch, however, as active growth

TABLE 3.—Loss in oven-dry weight of inoculated blocks under various moisture conditions during a 6-month incubation period, with paraffined-cork stoppers, except as otherwise noted

Loss of weight,	Moisture per c		Method
per cent	Original	Final	
0	39 39	8 6	Small inoculum
ŏ	45	39	Deep 3% agar gel, glass plate
ŏ	48	34	2 ml. 1.5% '' '' ''
ŏ	51	36	2 ml. 1.5% " " " "
Ö	$7\overline{2}$	9	Small inoculum
0	80	50	1 ml. 1.5% agar gel, glass plates
0	97	74	1 ml. 1.5% " "
0	110	97	0.5 ml. 1.5% " glass plate on tube walls
0	117	89	1 ml. 1.5% " "
5	66	7	Deep 3% ""glass plate; cotton-and- rubber stopper
5	69	6	1 ml. 1.5% agar gel
5	74	6	1 ml. 1.5% " glass plate
5	79	69	1 ml. 1.5% " " " " "
5	95	26	0.5 ml. 1.5% " " on tube walls
5	102	37	0.5 ml. 1.5% " " " " " " " " "
5	146	33	Deep 3% " " cotton-and-
			rubber stopper
5	153	19	Small inoculum; soda lime (cotton)
5	190	8	(grass woor)
10	70	7	Deep 3% agar gel, glass plates; cotton-and- rubber stopper
10	81	6	1 ml. 1.5% agar gel
10	108	34	glass plates, cotton-and-
10	108	31	rubber stopper Deep 1.5% agar gel, " " "
10	114	32	rubber stopper Deep 1.5% agar gel, " " " "
10	117	7	2 ml. 1.5% agar gel, "
10	126	39	Small inoculum
10	136	37	Deep 3% agar gel, glass plate; cotton-and- rubber stopper
10	154	97	2 ml. 1.5% agar gel, glass plates
10	192	146	2 ml. 1.5% "" "" ""
14	49	36	1 ml. 1.5% " " " "
14	138	76	2 ml. 1.5% " " " "
14	148	69	2 ml. 1.5% " " " "
15	54	43	2 ml. 1.5% " " " "
15	135	162	Deep 1.5% " " cotton-and-
75	197	233	rubber stopper
15	137	⊿ 55	Deep 1.5% agai gei,
16	108	66	rubber stopper 1 ml. 1.5% agar gel, ""
16	118	49	Small inoculum
16	130	167	Deep 1.5% agar gel, glass plates; cotton-and-
and a nd and a		la Jina	rubber-stopper
19	99	32	1 ml. 1.5% agar gel
21	128	286	Deep 1.5% agar gel, "" "" "" ""
30	131	161	Deep 1.5% agar gel, "" "" "" ""
31	131	227	Deep 1.5% agar gel, "" "" ""

of the test fungus was obtained under a number of conditions, it will be of interest to present a few of the observations. These are given in tables 3 and 4.

TABLE 4.—Showing conditions under which abortive sporophores were produced during a 6-month incubation period, with paraffined-cork stoppers

Moisture per c		Loss of weight,	${f Method}$
Original	Final	per cent	
43	14	4	Small inoculum
54	35	7	
64	39	7	
81	6	10	1 ml. agar gel
83	14	11	1 ml
85	7	12	1 ml. " " glass plate
86	31	17	1 ml. " "
89	41	25	1 ml. " "
92	37	13	1 ml. " "
99	46	23	1 ml. "
99	32	19	1 ml. " "
117	7	7	2 ml. " " " "
118	49	16	Small inoculum

SUMMARY

This paper describes results of a study of suitable methods to use in investigating the relation between moisture content of wood and the activity of decay-producing fungi. In this study *Fomes roseus* was grown on sapwood of *Pinus ponderosa* at a temperature of about 27° C. for approximately 6 months.

By adding water to test blocks in a stender dish, autoclaving them the following day at atmospheric pressure, and allowing them to stand another day, uniform moisture distribution was obtained. No method, however, was discovered whereby the desired amount of moisture could be added to a test block, except approximately.

The use of small bits of mycelium gave indications of being a satisfactory method of inoculating test blocks.

Tests of methods of stoppering culture tubes have progressed to a fairly satisfactory point. The use of paraffined corks promises good results, but more study is still needed on this problem.

The autoclaving incident to the process of adding moisture to the test blocks proved a satisfactory method of sterilization for the test materials used and under the conditions of experimentation adopted.

These studies failed to yield specific information on the relation of moisture content to activity of wood-destroying fungi.

CARMEL, CALIFORNIA.

VARIETAL SUSCEPTIBILITY OF BEANS TO AN AMERICAN AND A EUROPEAN STRAIN OF PHYTOMONAS MEDICAGINIS VAR. PHASEOLICOLA, AND A COMPARISON OF THE STRAINS IN CULTURE

WALTER H. BURKHOLDER AND KAROL ZALESKI1

Although the disease of beans caused by *Phytomonas medicaginis* var. *phaseolicola* Burkh. has been known for only 5 years, it is already recognized as the most serious bacterial trouble of this crop. Burkholder (3, 5), Hedges,² Higgins (6), and Zaumeyer³ have described its severity of attack in various sections of the United States. Furthermore, the disease is not limited entirely to America but, in Europe, it has been reported from Germany by Stapp and Kotte (9), from Holland by Wieringa (10), and from Switzerland by Burkholder (5).

While the severity of the disease has been emphasized by the abovementioned American investigators, little has been written concerning the various degrees of susceptibility that our American commercial varieties of beans exhibit toward the trouble. It has been observed in New York that a definite amount of resistance could be found in certain varieties, and Higgins (6) has reported this to be true in his work with 10 varieties of beans in Georgia. More extensive varietal-susceptibility tests have been conducted in Europe by Wieringa (10) and more recently by Kotte (7). The varieties of beans used by these two European investigators, however, are not grown in America, so that their published results are not very helpful to workers on this side of the Atlantic.

For some time it has been felt that further knowledge concerning the varietal susceptibility of American commercial beans of both the dry-shell and snap varieties was desirable. Such information could be used not only in extension work among the growers but as a basis for breeding work to secure desirable resistant varieties. The present investigation is an outcome of this need. The work reported in this paper, however, is not limited entirely to this phase of the disease problem but is coupled with the

- ¹ Adjoint in Botany and Plant Pathology, University of Poznan, and Fellow on the Fund of National Culture under the President of the Council of Ministers at Warsaw, Poland.
- ² Hedges, Florence. Bacterial diseases of beans in some western commercial seed-growing and canning areas and southern trucking sections in 1927 and 1928. U. S. Dept. Agr., Bur. Plant Indus. Plant Dis. Rptr. 12: 121-122. 1928.
- ³ Zaumeyer, W. J. Bean diseases in western United States in 1929. U. S. Dept. Agr., Bur. Plant Indus. Plant Dis. Rptr. 14: 38-43. 1930.

problem of whether or not the American and European strains of *Phytomonas medicaginis* var. *phaseolicola* are identical.

METHODS OF INOCULATION

Before proceeding directly to the varietal-susceptibility tests, a discussion is pertinent here as to the most satisfactory method of infecting the bean plants with the pathogen under consideration. Such a method should be one in which the severest and most uniform infection could be secured. The senior writer had experienced some difficulty during the previous season in obtaining uniform infection on varieties in the field, so, in this case, greenhouse trials were decided upon. Maturing seedlings or young plants approximately 15 cm. in height were used and three methods of inoculation were tried in an extensive series of experiments. It should be stated that these comparative infection tests were run at the same time that two of the main varietal susceptibility experiments were conducted. They are treated separately here only as a matter of convenience. Thirty-four varieties of beans were used in these tests and are the first 34 listed in table 1. were run in duplicate, since two different strains of Phytomonas medicaginis var. phaseolicola were used, and, furthermore, the entire experiment was repeated. Thus, a sufficient amount of data was obtained from which to draw fairly adequate conclusions on the methods used.

Each of the 34 varieties was divided into 3 groups of approximately 4 plants each. One group of plants was inoculated by spraying with a water suspension of the bacteria, while the plants in the second group were injured at the cotyledon node and the inoculum inserted into the wound. The third group was injured and inoculated at the first leaf node. In the methods where the plants were injured the procedure as described by Burkholder (5) was followed. In all cases the pathogen was grown on agar slants at 24° to 27° C., and 2- to 3-day-old cultures were used. When the plants were inoculated by injuring them the bacteria were removed from the agar on the tip of a sharp pointed scalpel and the plants were stabbed with this contaminated instrument. In these trials no special care of the plants was taken; that is, they were allowed to remain on the greenhouse benches before and after inoculation. When the plants were inoculated by spraying with a water suspension of the bacteria from agar slants they were first placed in a moist inoculating chamber for 24 hours, as recommended by Smith and others, and, after spraying, were again placed in the inoculating chamber. One group of plants was allowed to remain under these moist chambers for 24 hours after inoculation and a second group for 48 hours.

The first of these comparative experiments was conducted in the green-house in July, 1930, and the second set in August, same year. Under the

TABLE 1.—Showing varietal susceptibility of American commercial varieties of beans to two American strains and one Euro-pean strain of Phytomonas medicaginis var. phaseolicola

	First	First trial	Secon	Second trial	Thir	Third trial
Varieties	1st American strain	European strain	1st American strain	European strain	2nd American strain	European strain
Black Valentine Bountiful Burpee's Kidney Burpee's Stringless Currie Davis Wax Dwarf White Kidney French Horticultural Gilant Stringless King of the Barlies Longfellow Low Champion Pencil Pod Black Wax Pod Spot Proof Wax Refugee Wax Red Kidney Red Valentine Red Valentine Roger's Stringless Refugee Roger's Stringless Refuge Roger's Stringless Refuge Roger's Stringless Refuge Roger's Stringless Refuge Roger's Stringless Round Pod Seotia Simmons Stringless Round Pod Suressee Green Pod Unrivalled Wax	Moderate Severe (' Severe Very Slight Severe Moderate (' Severe Moderate Very Slight Severe Very Slight Severe Moderate Very Slight Moderate Very Slight Severe Moderate Very Slight Severe Very Slight Moderate Slight Moderate Very Slight	Slight Very Severe Severe (, , , , , , , , , , , , , , , , , , ,	Very Slight Moderate Slight Very Slight Moderate Very Slight Very Slight Slight Slight Slight Severe Very Slight Slight Severe Very Slight Slight Severe Very Slight Slight K	Slight Very Severe Moderate Severe Moderate Severe Moderate (',',' ',' Very Severe ',' ',' ',' Very Severe Slight Moderate Slight Moderate Slight Worly Severe Slight Very Severe Slight None Severe None	Very Slight Sight Sight Severe Sight None Slight Severe Moderate Very Severe (',' ',' Slight Very Slight ',' Suight ',' Sight ',' Sight ',' Severe ',' Slight ',' Sight ',' Severe ',' Slight	Very Slight Moderate Very Slight Moderate None ('' Slight '' Kone Slight '' None None Very Slight None Very Slight '' Severe None Very Slight Moderate None None None None None None None Non

TABLE 1.—(Continued)

	First	First trial	Secon	Second trial	Thir	Third trial
Varieties	1st American strain	European strain	1st American strain	European strain	2nd American strain	European strain
Wardwell's Kidney Wax Webber Wax White Marrow White Kidney Yellow Eye Blue Pod Medium Challenge Black Wax Full Measure Kentucky Wonder Lazy Wife Perry Marrow Otenashi Robust, Pea	Severe () Moderate Very Severe Moderate	Very Severe Severe '(r Very Severe Severe	Moderate ''' Very Slight Moderate Slight	Very Severe Severe Very Severe Slight	Very Severe '(' '(' Severe Very Slight None Slight Severe None Severe None Severe None Severe None Severe	Moderate Slight Moderate Slight Very Slight None Slight None Severe Severe Severe None Sight None Sight None Sight None Sovere Sovere None Sight
Well's Red Kidney					very Severe	". Moderate

1932]

conditions of the experiments the results demonstrated that stabbing and inoculating the plants at the first leaf node gave a more consistent and a greater degree of infection than the other methods employed. This was evident not only on those varieties showing an extreme susceptibility but also on those that displayed a certain amount of resistance. It was to be expected that the disease would progress more rapidly in the plant and become more severe when the organism was introduced directly into the xylem. It is in this tissue that the invasive ability of the bacteria is greatest. When the bacteria were sprayed on the leaves their entrance through the stomates and establishment in the tissue were somewhat slower. Also, a further disadvantage of the spray method, one of greater importance for a varietal susceptibility test, was the fact that infection was not uniform even among individuals of the same variety. The causes of these irregularities probably are many, but an uneven distribution of inoculum, no doubt, is a primary factor.

Infection took place more quickly and the symptoms of the disease became more severe when the plants were inoculated at the first leaf node than at the cotyledon node. This difference in degree of infection seems due merely to the position of the inoculation court. The first leaf node was more centrally located.

Some attempt was made to conduct comparative infection experiments on the pods, but the data collected were few and are not given here. The pods of the different varieties were seldom produced at the same time; consequently, the results were not comparable.

VARIETAL SUSCEPTIBILITY TESTS

In conducting experiments to determine the varietal susceptibility of the more common commercial varieties of beans 3 different tests were made. The first experiment was carried on in July; the second, in August; and the third extended from the middle of October into November. The temperature during the summer months was variable and at times was as high as 35° C. at noon. During October and November the greenhouse in which the experiments were conducted ranged from 25° to 29° C. This fairly constant temperature held in the last experiment was maintained through the use of automatic devices installed in the greenhouse.

Thirty-four varieties of beans were used in the first 2 experiments, but 9 additional varieties and 1 duplication were added in the final experiment. In the first test the beans were grown in flats (45 x 32 cm.) in 3 rows of 8 each, but in the following experiments 6-inch pots were used. The last method was more satisfactory, since the plants had more soil and made a better growth. The number of plants of each variety inoculated varied

somewhat due to the fact that at times there was a poor germination of seed. Seldom, however, were there less than 8 plants in a variety, and in the majority of cases there were 10 or 12. This number does not include the plants inoculated by spraying and those inoculated by stabbing at the cotyledon node. Only those inoculated by injury at the first leaf node were considered in the varietal-susceptibility experiments.

Since the trials were conducted 3 times, approximately 25 individuals were used in each of the 34 varieties. The number of each of the 10 varieties added in the last experiment was 8 to 12.

In the first 2 sets of experiments 2 different strains of *Phytomonas medicaginis* var. *phaseolicola* were used, one of American and the other of European origin. The latter strain was obtained through the courtesy of K. F. Wieringa, who had isolated it from material collected in Holland. The American strain was isolated from a Red Kidney bean pod collected in New York State in 1924, but it had been inoculated repeatedly into bean plants and reisolated since then. Both strains appeared to be virulent. In the third trial the Holland strain was still used but the American strain referred to above was discarded for a second strain isolated from bean leaves collected in New York in September, 1930.

The plants were inoculated usually about 10 to 14 days after planting or when they were approximately 15 cm. in height. Three weeks after inoculation the plants were carefully examined to determine the amount of infection and the data for all 3 sets of experiments are recorded in table 1.

This time of examination was not chosen arbitrarily but through careful observation. The progress of the disease on the young plants was watched from the time of its appearance until a month or so had elapsed after inoculation. At the end of three weeks degrees of infection were, as a rule, very distinct but, after this time, there was a mingling of certain adjacent degrees.

In determining the degree of susceptibility of each variety a method similar to that used by Barrus (2) and by Rands and Brotherton (8) was employed. Six classes or degrees of infection were adopted and are described below. A description of the symptoms of the disease is not given here, since they have been described in full, with illustrations, by the senior writer (5) and a repetition appears unnecessary. Following are the degrees of infection.

None. There is no evidence of any symptom of disease or only numerous small local lesions appear.

Very slight. Small lesions of sufficient number do at times cause the death of a single leaf.

Slight. The first leaves are destroyed but the plant appears to outgrow the infection and at the end of 3 weeks there is little evidence of the disease.

Moderate. Systemic infection is evident. A moderate stunting of the plant and at times chlorosis of the leaves take place. The lower leaves, as a rule, are lost.

Severe. The symptoms due to a systemic infection are very evident. Plants are stunted approximately 33½ per cent. Leaves are dwarfed, chlorotic, and wrinkled. At the end of 3 weeks plants are alive, although there is no promise of even a moderate crop of seeds.

Very severe. Plants are dead 3 weeks after inoculation.

In examining table 1 it may be seen that there is some variation in the degree of susceptibility of a variety occurring in the different trials and between the different strains, even in the same trial. These variations are never consistent, however, and may be explained here on the basis of environmental factors and differences in virulence of the organisms. Certain of the trials and varieties should be commented upon, however. In the first trial the infection, on the whole, appears to be slightly more severe than in trials two and three. This apparent difference is due possibly to the fact that these plants were growing in flats with little soil and in an environment where the tendency was for the soil to become dry and com-Thus, the plants were weakened and infection appeared greater than it probably really was. The American strain used in the third trial was recently isolated and appeared at times somewhat more virulent than the strain used in trial two. On the whole, however, no consistent difference can be seen between the reactions of the varieties of beans to the American strain and to the European strain of Phytomonas medicaginis var. phaseolicola. Those varieties that show a great susceptibility to one strain also exhibit this characteristic to the other strains.

Considering the table as a whole, one can readily point out the varieties of bean that are very susceptible to the disease and those that show a great amount of resistance. Since there were three trials and several strains of the organism used, the data should yield results of a fair degree of accuracy.

The varieties of beans that are very susceptible to the disease are as follows: Bountiful, Giant Stringless, Improved Golden Wax, Longfellow, Low Champion, Red Kidney, Ruby Horticultural, Simmons Stringless, Round Pod, Unrivalled Wax, Wardwell's Kidney Wax, Webber Wax, White Kidney, Lazy Wife, and the Well's Red Kidney.

Those varieties that show a great amount of resistance to or tolerance for the disease are: Black Valentine, French Horticultural, King of the Earlies, Pencil Pod Black Wax, Pod Spot Proof Wax, Refugee 1000-1, Refugee Wax, Rogers Stringless Refugee, Round Pod Kidney Wax, Scotia,

Sure Crop Wax, Tennessee Green Pod, Blue Pod Medium, Challenge Black Wax, Kentucky Wonder, Otenashi, and Robust Pea. The 12 remaining varieties probably should be referred to as moderately susceptible.

The majority of those varieties listed above as very susceptible to Phytomonas medicaginis var. phaseolicola have been tested also for their susceptibility to Phyt. phaseoli by Burkholder (4) and by Rands and Brotherton (8). In these two latter reports this group of varieties also appears to be very susceptible to Phyt. phaseoli. One would think there was a correlation between susceptibility to the two diseases. hand, however, the varieties of beans found by the writers to be but slightly affected by Phyt. medicaginis var. phaseolicola may or may not be very susceptible to Phyt. phaseoli, and the correlation that appeared in the first group is not found in the second group. It is true, nevertheless, that such common bean varieties as the Refugee 1000-1, Roger's Stringless Refugee, Robust Pea Bean, Scotia, and French Horticultural show resistanance to both organisms, but this rule does not follow with certain of the The Black Valentine may be cited as such an example. Both Higgins (6) and the writers list this variety as only slightly affected by Phyt. medicaginis var. phaseolicola, but Burkholder (4) and Rands and Brotherton (8) state that it is severely affected by Phyt. phaseoli. condition also is similar to that in certain other varieties.

Wieringa, in his work on varietal susceptibility of beans to the disease, found a correlation between susceptibility and purple blossoms. Kotte (7) did not observe this correlation, and it was not found by the writers in this present investigation.

COMPARISON OF THE VARIOUS STRAINS OF THE PATHOGEN IN CULTURE

Although the varietal susceptibility experiments reported above indicated that the European strain of *Phytomonas medicaginis* var. *phaseolicola* is identical with the American strains, at least on a pathogenicity basis, it was considered worth while to make a comparative test of the organisms in pure culture. In conducting these tests with the 3 strains of the pathogen used above, it soon was apparent that they coincided in appearance and in reaction in the various media. The morphology of each was similar, as also were their growth characteristics in such common media as beef-extract bouillon and agar, milk, and Uschinsky's solution. There was a slow liquefaction of gelatin, nitrates were not reduced, indol was not formed, and there was only slight, if any, hydrogen sulphide produced. All 3 strains were facultative anaerobes, as shown in dextrose broth in fermentation and in shake agar cultures.

Extensive carbohydrate-fermentation studies were conducted with the hope of finding differences between the strains, but the results were negative. Since the majority of substances used in these experiments, however, have not been reported upon before for this organism, they are given here in some detail. In the main, the basic medium used was the modification of the solution used by Ayers, Rupp, and Johnson and recommended in the Manual of Methods for Pure Culture Study of Bacteria issued by the Society of American Bacteriologists. The bacteria grew well in this simple solution, showing that they could use inorganic nitrogen; and, since the carbohydrate to be tested was the only source of energy for the organism, the determinations of the fermentation of the substance were not complicated. Brom-Creosol-purple was used as an indicator, and all carbohydrates were sterilized by autoclaving with the exception of lactose, maltose, raffinose, and sucrose. These 4 sugars were filtered. The recommendations of Ayres, Rupp, and Johnson (1) were followed in preparing the organicacid media. In the fermentation of starch the starch-agar-iodine method was used.

All 3 strains fermented the following carbohydrates but without the production of gas; dextrose, levulose, galactose, mannose, arabinose, xylose, sucrose, glycerol, citric acid, and malic acid. The following were not fermented; rhamnose, lactose, maltose, raffinose, mannitol, salicin, starch, acetic acid, benzoic acid, lactic acid, and tartaric acid.

SUMMARY

Experiments were conducted to determine the varietal susceptibility of a number of American commercial varieties of beans to the disease caused by *Phytomonas medicaginis* var. *phaseolicola*. Two American strains and one European strain of the pathogen were used. There were no differences in the susceptibility of varieties to the different strains. Certain varieties, however, proved to be considerably resistant to the disease, while others displayed various degrees of susceptibility.

The European strain of the pathogen was compared in pure culture with

the American strains. No differences were noted.

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IMMUNITY IN PLANTS

J. A. DE TOMASI1

While we may find it easy to describe all of a series of immunological facts, we may not find it so easy to agree on a definition of immunity. Immunity, as we understand it, is applied to that animal that does not contract an infectious disease to which it is susceptible even though it comes in contact with the microorganism or the agent of the disease. Thus, it is evident that we are considering only the pathogenic power of the agent rather than the faculties of the agent to enter into a symbiosis, either mutualistic or parasitic. In animals illustrations of this sort are frequent, not only in their open organs that communicate with the external world directly, as the intestines, or, indirectly, as the cystiphellea, but also in their closed organs, such as the circulatory system. In the case of healthy carriers the microorganisms, pathogenic and virulent in themselves, are harbored for long periods without evincing any apparent harm. Common examples applicable to open organs of the human carrier are: bacilli of tuberculosis and of diphtheria in the respiratory tract; vibrios of cholera; and typhoid bacilli in the intestinal tract. On the other hand, one finds the treponema of syphilis practically anywhere in man, and, in 1923, De Kock informed us from South Africa that horses can carry the virus of pernicious anemia in their blood as long as 7 years after the cure (10).

The above concepts are useful and practical in the field of plant immunology, as well as in medical bacteriology. In fact, one should make evident that some of the most characteristic examples of immunological reactions in plants are drawn from the chapter on symbiosis, not, however, from the parasitic but from the mutualistic. It is for this reason that the Italian school has introduced the term refractoriness, to indicate the absolute impossibility of any relationship of life in common and to mean by immunity the act of hindering the symbiont from becoming pathogenic.

Among the forms of immunity that we are accustomed to distinguish there are, primarily, general and local immunity. One of the chief objections to phytoimmunity is that in plants one cannot think of an immunization of the whole organism because of the great independence of their parts.

Before stating the facts that answer the objection, it will be opportune to note at this point that most recently we have learned more and more to acknowledge and to evaluate in just terms the importance of strictly local immunizations, even in animals. Citing a typical case, we have the work of Altana, who infected the cornea of a rabbit with smallpox vaccine (1). After the healing of the specific lesion, the affected part enjoyed an out-

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Before stating the facts that answer the objection, it will be opportune to note at this point that most recently we have learned more and more to acknowledge and to evaluate in just terms the importance of strictly local immunizations, even in animals. Citing a typical case, we have the work of Altana, who infected the cornea of a rabbit with smallpox vaccine (1). After the healing of the specific lesion, the affected part enjoyed an out-

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standing immunity not at all shared by the rest of the body, which continues in its own state of susceptibility. The establishing of a general immunity, on the other hand, does not protect the cornea from being susceptible to successive vaccinations.

Another important objection is based on the fact that the fluids of plant cells are generally acid, while those of animal cells are alkaline. It is claimed that this difference is so fundamental that we can not assume any analogy between the behavior of plant and animal cells. However, acidity and alkalinity are only relative terms. The pH of animal fluids varies over a wide range and is only a continuation of the range of pH covered by plant juices. Lately, the study of immunological facts of the rudimentary animals seems to have resulted in extremely interesting observations of the few who have ventured into this field, Cantacuzene and Metalnikoff (7, 14). We find statements of irrefutable value where imperceptible steps are established by endless gradations from the animal to the vegetable realms. More time should be spent on the interesting chapter on immunology in Protozoa and Metazoa up to the insects, but, regretfully, space permits but a mere mention of them. Note the single case of that Tunicata, Ascidia mentula, which is an animal whose blood has an acid reaction like plants.

As for the plants, we all know that the most exact knowledge of the degree of resistance of cultivated plants to the most serious diseases is of a very practical value, chiefly in relation to the variability of resistance of singular races. This makes possible a choice among them, according to the environment, and it can constitute, furthermore, one of the cardinal points in the perfecting of artificial selection. However, we know a great deal less about the nature and mechanism of congenital immunity in plants. tainly theories have been formulated, but all of them sin in one-sidedness, either because they seem tentative in simple adaptation through analogy with animal immunology or because they are based upon isolated specific observations that do not yet lend themselves to generalizations. If we consider essentially the distribution of immunity in plants and the principal fact of the specialization of parasites, the factors of resistance in plants can be thus classified: (1) Mechanical defenses dependent upon the anatomical structure of tissues. (2) Actions of normal cellular and intercellular fluids before the parasitic attack. (3) Histogenic and humoral reactions of tissues under the action of the parasites.

From 1911 to 1919 Vavilov (19) thoroughly studied the question of the distribution of immunity and its connection with the specialization of the parasite. He concluded that the more limited the field of receptibility of a given fungus, the easier it is for this to be susceptible to the different reactions of some varieties; the more likely it is to find varieties in the same species that are immune or at least much less susceptible. Sclerotinia tri-

foliorum Eriks. attacks Medicago, Onobrychis, and some other genera of the Papilionaceae; it is very little specialized and consequently no varieties of immune clover are observed. But the rust known as Melampsora lini Boll. is incapable of affecting anything other than the individuals of the genus Linum. Accordingly, there really are varieties of flax perfectly resistant to this rust.

Without considering the subtle distinctions among nonspecialized parasites in the so-called physiologic forms, where many points are still uncertain, Stakman and his collaborators distinguish approximately 100 physiological forms of *Puccinia graminis* Pers. in this country (18). It is worth while, however, to call attention to the fact that the distribution of immunity in a single given species is particularly related to the genetic differentiations of the varieties.

Triticum monococcum L., a species of wheat, resists in all its varieties the brown, the yellow, and the black rusts—a fact that distinguishes it particularly from any other wheat. Genetically, Triticum monococcum occupies a place distinct among wheats; and it does not yield fertile hybrids with other species.

Passing in rapid review the mechanical factors of congenital immunity, we find it important to mention the specific action of plant juices. Furthermore, we also find it rather easy to understand the functioning of the hundreds of means of defense exploited by nature from the number of stomata on the leaves and the different movements of stomatal cells to the infinite forms of flowers and to suberifications of the cortical cells and to the thickness of the sap.

The natural defense is based on a few well-known elements and on many not so well known; and it still represents a magnificent field for investigations. The Americans have of late devoted a great deal of attention to it; their collected data have value of noteworthy significance.

The organic acids more or less diffuse in plants can inhibit parasitic growth; Bacillus carotovorus L. R. Jones infects the tomato quite easily but does not attack oranges or lemons. Nobécourt has proved and stated this fact in vitro, using sterile extracts, raw and boiled, of the above-mentioned fruits (15). The action is identical with that of a solution of citric acid of the same concentration, but, thus considered, the assertion seems too obvious, because probably we must know better the single physiological activities of the present substances before formulating a possible definite conclusion of a general character.

In fact, Hursh, among others, in a study of a large number of wheat plants, observed that a parallelism exists between resistance and acidity as expressed in terms of pH (13). Anthocyanines among pigments are supposed to act as inhibitory substances by virtue of the fact that they are derived from bodies endowed with a high osmotic power.

The alkaloids are inactive, just as are the essential oils, but the latter are quite active *in vitro*. There are, in addition, some investigators who have observed phenomena attributable to true immunological factors. Wagner is one of them. He cites three groups of antibacterial principles (20): (1) The agglutinin that would hinder the movement of cilia. (2) The lysin that would dissolve the membranes of the infecting organism. (3) Other substances, inhibitory to germination of spores.

He also states that the bactericidal factor is strictly connected with the protein fraction of the sap and that it is destroyed by heating 15 min. at 60° C., by oxidases, and peroxidases.

However, let it not be forgotten that constancy and specificity of the reactions are extremely variable. At all events this field seems open to brilliant research work to whoever plans to devote himself with systematic directions. Undoubtedly it is within the microchemical and hystogenic reactions of sap in parasitized plants that we should expect a new light.

So far we have considered only the natural factors of resistance of plants. Let us now turn to the condition of the plant when a host. have then symbiosis of all possible types, from the most harmless up to the occasionally encountered violent forms where tissues build up physical barriers or where thickly invaded leaves, flowers, or branches are actually shed. Reactions of a physiological nature become evident by the diminishing weight of the dry substance in the diseased leaves through the rise of temperature, as Pavarino says (16), just as in the case of fever in animals. Furthermore, we should recall at this point the theories of Malpighi originally formulated in his classical work, "De Gallis." These theories found favor with a number of Italian and other foreign phytopathologists and were subsequently taken up by Sachs and Beyerinck. To-day there are generally accepted hypotheses of a certain quid that stimulates growth as well as cicatrization in plants. Apparently these principles have numerous points of contact with animal hormones or secretions of the endocrine glands. Finally, there has been studied in multiplex forms the phenomenon of phagocytosis of vegetable cells occurring in the roots of many plants, e.g., Orchis. In these cases the germination of seeds is subordinate to the infection brought about by the respective Rhizoctonia. Bernard, using different mycorhizas instead, was able to point out that, while some embryos succumb to the invasion of the mold, others react by means of their cells, limiting the activity of the fungus to a minimum sufficient to guarantee a symbiosis: that is to say, an equilibrium which Bernard calls "à la frontière de la maladie" (5).

In addition to these, there is a third case in which the mycelium of the parasite is actually destroyed. The invading hyphae penetrate the embryonic tissues, assume a vegetative character opposed to rapid diffusion,

and proceed to the formation of a clew within the single cells. In the case of the resistant Orchis this clew of mycelium is successively attacked and destroyed. The nucleus of the cells hypertrophies, assuming at times amoeboid aspects; it winds its way through the mycelial mass, resulting in a degeneration of the clew into an amorphous complex; it is sometimes capable of returning very slowly to the primary form. As for the hypothesis of autolysis, one tends to-day to substitute in these cases the interpretation of active cellular factors that operate as directly as the aforementioned nucleus.

The abnormal manner of development of the endophyte within the plant can be related to the phagocytosis, on the one hand, and to the humoral properties of the saps, on the other. This degree of immunity is comparable to that of vaccinated animals.

Indeed, from the tubercles of Orchis, Bernard (6) succeeded in preparing an extract with specific fungicidal and coctible character at 55° C. for 35 min. In concluding what occurs within the Orchis, it is interesting to remember that cellular reactions of a very similar type have been noticed by Demkerly in the cells of a worm in respect to a coccidium. Not only have analogous statements been gathered together recently in the physio-pathological study of several other cases of plant infection in Europe but also a very instructive case has been found on Arisaema triphyllum (L.) Schott., a common plant of the woods of New York (11).

To conclude the subject of congenital immunity, let us recall that in cases of wheat plants, subject to *Puccinia graminis tritici* Eriks. & Henn., and of flax, subject to *Fusarium lini* Boll., the presence of immunological factors is made evident under the forms of the so-called hypersensitive reaction characterized by a rapid spreading of the infection, which induces a virulent histological and humoral reactivity that is thoroughly capable of checking the parasite (21).

We now come to the subject of acquired immunity through plant vaccination. Many objections to this have been based on several facts, such as the anatomical and the physiological differences between plants and animals, the usually localized type of vegetable infection, and the supposed lack of a circulation comparable to the circulation of blood in animals, where the immunological phenomena have their principal seat. The circulation of saps is still, for many people, simply a mechanical fact that concerns chiefly the mere lignified vessels without any possible intervention in the delicate processes as associated with immunity. But the most modern views on plant pathology tend to emphasize the meaning of any living cell in the circulation of saps. Concerning the type of infection, let it be sufficient to recall that there are infections in plants due to bacteria that infest the whole organism; also, there are some fungi that spread through cribriform vessels. And do not the most recent researches on animal cells cul-

tured in vitro show that they are still capable of producing specific antibodies? There are here, then, no circulatory or nervous correlations; in other words, conditions of life are entirely similar to those of vegetable cells.

Moreover, we can not ignore the importance of the recent studies of Gioelli (12) on the variations of vegetable saps in diseased plants. By means of both chemical and physicochemical analyses and by an extensive application of the refractometric methods, he was able to prove that proteins in diseased vegetables undergo variations analogous to those already observed in diseased animals. Gioelli concluded that "the plant cells react to the infection as the animal cells."

Let us now consider some specific case of vaccination as it has been experimented upon till this very day by several workers. Though these experiments are few and not at all free from criticism, they reveal a definite accomplishment of positive results. Simply on the basis of these first encouraging experiences, we hope for increased interest in this very new and promising field of investigation whose prospects are undoubtedly fruitful.

Carbone (7) lists in a recent paper 18 cases of phanerogamic hosts and cryptogamic parasites whose aspects of immunological phenomena have been checked by various authors. Let us select a few. In 1901 Beauverie (3) observed that when very young Begonia plants are cultivated in the presence of a strain of a weakly-virulent parasite, they resist the attack of Botrytis cinerea Pers. Shortly after, Ray (17) stated that when lupines, beans, wheat, and oats are treated previously, either with attenuated cultures of the microorganism or with its extracts, they resist Bacillus putrefaciens Ray, which is the common cause of rot, and become slightly diseased for a short time, after which they remain immune from succeeding infections. Zoja (22) obtained similar results by vaccinating wheat against Helminthosporium sativum P. K. & B. Acquired immunity was realized by letting the seeds germinate on the aqueous extracts of parasite cultures. After infection, these plants did not show the fungus in any cell. On the other hand, plants germinating in pure water certainly revealed the fungus.

Under different conditions Arnaudi (2) experimented with potatoes by means of a simple technique and achieved similar results. On each side of the potato two cup-like cavities were made, one moistened with the attenuated broth culture of *Bacillus mesentericus* (Flügge) Mig., the other with sterile broth. After 24 hours, at 30° C., Arnaudi scarified both cavities to break the subereous layers and placed in each a loop of the virulent cultures. With variations, dependent upon the degree of the attenuation of the immunizing material, where it has been used, the tissue remained either untouched or only slightly attacked, characterized by a dry circumscribed area not at all comparable to the imposing work of destruction noticeable in the other cavity. In some other work on *Pisum sativum* L., the same author

has shown not only a greater acquired resistance by the prolongation of life of the vaccinated subjects but also the specificity of the immunizing action; in fact, the parallel vaccinations with Aspergillus oryzae Ahlb. Cohn and Blepharospora cambivora Petri behave quite differently. When the young plants are artificially infected only by the Blepharospora itself they will show a distinct resistance to it, while those vaccinated with the Aspergillus do not reveal any specific resistance to the Blepharospora.

Several other examples can be added to these, such as those recently published by Benigni (4), in Italy, and Nobécourt (15), in France.

The results on the vaccination of plants should be brought together. While the Italian and German workers confirm the presence of a state of high resistance to the infection, the French authors affirm having achieved complete immunity in reinoculation. As far as we know of cases to date, the state of immunity does not seem to last much longer than 1 month. Concerning the nature and the properties of antigens, the first attempts in this field seem to indicate many capital points of contact with the animal antigens, themselves. It is obvious, however, that much, if not the entire field of this specialty, yet remains to be investigated. At all events it seems that we can already detect a decisive action, either of histogenic or of humoral factors.

Finally, there is to be considered the question of superinfection. When a more or less latent infection occurs in an organism, and this is again attacked by the same infectious agent in the same or another part of it, we are referring to superinfection. It may happen that the organism does not react at all to the second infection. This property is distinguished as resistance to the superinfection, and it is essentially one of the types of acquired immunity. Now, the same immunity has been checked, even for plants. Arnaudi, to mention a single name only, working on geranium plants with *Bacillus tumefaciens*, concluded that, out of 13 inoculations into subjects with preexistent tumors, 9 were healed; 4 formed simply tiny tumefactions. The control on 9 inoculations on healthy plants resulted, instead, in 7 cases of tumors and 2 negative cases (2).

There still remain many points to be considered in relation to immunological phenomena in plants. He who desires to increase his knowledge in this field of study, which, furthermore, concerns passive immunity, humoral factors, bacteriophagy, anaphylaxis, and experimental and applied technique, should consult the recent monograph on plant immunity by Carbone and Arnaudi (9). Because of its very thorough discussion on any subject in this field, and of its extensive bibliography, this work is of major importance to that biologist who seeks new perspectives in the study of the pathology of vegetables.

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PHYTOPATHOLOGICAL NOTE

A correction.—In our paper entitled "Stripe rust, Puccinia glumarum, on wheat in Argentina" (Phytopathology 20: 981-986, 1930) there appears on page 981 the following statement: "Elsewhere, in South America, it [P. glumarum] apparently had never been collected until October, 1929, when the junior writer [Richard O. Cromwell] observed it in . . . Argentina." Our attention has since been called to a published account of an earlier observation and identification of this rust in Argentina by W. Rudorf, who collected it also in October, 1929, evidently only a few days before it was observed by the junior writer.—Harry B. Humphrey and Richard O. Cromwell.

¹ Recientes análisis de trigos hacen prever una infección de "Puccinia glumarum tritici." La Razon, Agosto 22, 1930. Buenos Aires.

PHYTOPATHOLOGY

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PHYSIOLOGIC SPECIALIZATION AS A FACTOR IN THE EPIPHYTOLOGY OF PUCCINIA GRAMINIS TRITICI¹

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INTRODUCTION

The phenomenon of physiologic specialization within *Puccinia graminis* Pers. was definitely established by Eriksson (2) in 1894. Stakman and Piemeisel (17), in 1916, demonstrated that further specialization existed within *P. graminis tritici* Eriks. and Henn. Since that time, physiologic specialization has been clearly demonstrated both in *P. graminis avenae* Eriks. and Henn. (14) and *P. graminis secalis* Eriks. and Henn. (6).

The varieties of *Puccinia graminis* now known to be present on the North American Continent, with the degree of physiologic specialization within each variety, so far demonstrated, are as follows:

1. P. graminis tritici

Over 100 physiologic forms identified, about 60 of which have been found in nature in North America (13, 9, 15, and unpublished results by Stakman and Levine).

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- 2. P. graminis avenae
- 3. P. graminis secalis
- 4. P. graminis phleipratensis
 (Eriks. and Henn.) Stak.
 and Piem.
- 5. P. graminis agrostidis Eriks.
- 6. P. graminis poae Eriks and Henn.

7 physiologic forms identified; 5 have been found in North Amercan (14, 1, 3, and 18).

14 physiologic forms identified, all except one from North American collections (6 and unpublished results by Cotter and Levine).

Some indication of the existence of at least 2 distinct forms, according to unpublished results of Cotter.

Physiologic specialization not demonstrated.

No definitely established evidence of physiologic forms.

In the study of the epiphytology of stem rust in the United States a question of major importance is that of the spring source of inoculum. Studies over a long period of years continue to support the conclusion that urediniospores of *Puccinia graminis tritici* do not, in any important sense, survive the winters in the hard-red-spring-wheat area. What, then, is responsible for the occasional, rather general, and sometimes severe, epidemics of stem rust in this region? Is the primary inoculum still being produced in sufficient quantity on the remaining barberries to cause a general epidemic or is the migration of rust from southern areas the critical factor? It seems plausible that in the years when severe epiphytotics occur one or both of these sources may be important.

This paper reports primarily a study to determine the possible migration of stem rust from the South to the North. The study is based on the annual physiologic-form surveys of 1926, 1927, and 1928, supervised by the writer, who was stationed at University Farm, St. Paul, Minn., during these years. Surveys of this kind have been made each year since 1918 by Stakman and Levine.

Annual physiologic-form surveys show to a certain extent which forms occur in the South and which in the North. An indication is obtained of the prevalence and distribution of forms in these respective regions. The annual survey, then, should be one of the most satisfactory criteria in determining whether stem rust migrates from South to North.

It is obvious that knowledge of the existence of physiologic forms of wheat stem rust and of the prevalence and distribution of such forms is essential to the development of resistant wheat varieties.

The fact that new forms of *Puccinia graminis tritici* are rather frequently making their appearance or, at least, are being discovered empha-

sizes the importance of the surveys in the breeding of resistant wheat varieties. Again, if certain physiologic forms of *P. graminis tritici* are outstandingly predominant in a given region year after year, those forms are the ones to be primarily considered in a breeding program. It is important also to know if other forms occasionally occur in this particular region, even though they be comparatively uncommon. Further, it is of value to know as much as possible of the distribution of all existing forms. In spite of the fact that certain forms, isolated only from widely separated regions, seem relatively scarce, there is no reason to assume that these forms may not at some time in the future become generally distributed.

The surveys of 1926, 1927, and 1928 were similar to those made prior to 1926 by Stakman and Levine,³ and add certain information of interest and value to wheat breeders. The last three surveys, however, were made with the idea of studying particularly the possibility of the migration of rust from southern fields to those in the North. A careful analysis has been made of the data obtained, and this paper reports the conclusions reached regarding the source of inoculum in each of the 3 years and presents a discussion of the evidence for or against migration of stem rust of wheat from the South to the North.

EXPERIMENTAL MATERIALS AND METHODS

The methods used in making the surveys of 1926, 1927, and 1928 were similar to those used in the surveys made by Bailey (1), Levine (5), Newton, Johnson, and Brown (8), and Stakman et al. (13, 16). Collections of stem rust were obtained from the field and from uniform rust nurseries. Uredinial material was collected as early as possible in the spring and collections were continued until the disappearance of that stage in the fall. Collections from the uniform rust nurseries were made by the person who visited the nurseries to take notes on the rust infection of the wheat and oat varieties. The field material was collected by state leaders and scouts of the barberry eradication forces and by other rust workers. The rust was cultured and form identifications made in the plant pathology greenhouses at University Farm, St. Paul, Minnesota.

Differential hosts. The identification of physiologic forms of Puccinia graminis tritici is based on the reaction of 12 varieties of wheat that were selected by Stakman and Levine (13) and designated as "differential hosts." These 12 varieties are listed in table 1.

Inoculation and cultural methods. Upon receipt of uredinial material, inoculations were made as promptly as possible. When sufficient rust was available, the 12 differential varieties were inoculated from the original col-

³ Stakman and Levine—unpublished data.

⁴ Nurseries maintained cooperatively by the U. S. Department of Agriculture and various agricultural experiment stations over the United States and eastern Canada to test the reaction of wheat and oat varieties to stem and leaf rusts (5).

lection. However, in most instances it was necessary first to increase the inoculum by inoculating Little Club wheat, a variety susceptible to every form so far discovered in North America. The inoculation and incubation methods were essentially the same as those described by Stakman and Piemeisel (17). The seedlings of the differential varieties were grown in a secluded section of the greenhouse. When about 7 or 8 days old they were inoculated by applying a small quantity of urediniospores to the lower surface of each seedling leaf. A small flat needle was used for transferring the rust from the original material or increase plants to the differential varieties. An alcohol lamp was used for sterilization of the needle. plants to be inoculated were sprayed with an atomizer filled with tap water so as to induce the spores to stick to the leaves. Usually, the plants had not developed any secondary leaves when inoculated but, if secondary leaves were present, they were clipped off and only the primary leaves inoculated. Immediately after inoculation the plants were incubated for approximately 48 hours in large moist chambers. Upon removal from the chambers, the pots were placed in individual booths on the greenhouse benches. From 15 to 25 plants of each variety were inoculated. All plants not inoculated and those resulting from seed germinating after incubation were removed from the pot. The best possible precautions were taken at all times to prevent accidental infection or contamination.

Identification of physiologic forms. Readings could be made usually after 10 or 12 days, depending somewhat on the amount of sunlight during the developmental period of the rust. The types and degrees of infection described by Stakman and Levine (13) were used.

After all differential varieties were inoculated and had shown clearly their reaction to a particular collection of stem rust, the identity of the latter was determined by the use of the key shown in table 2. This key for the identification of physiologic forms of Puccinia graminis tritici is a revision of that of Stakman and Levine (13) and furnished by them. Many physiologic forms can be identified with this key without considering the reaction of the differential varieties. However, the reaction of all the varieties is always determined before deciding upon the identity of a particular physiologic form. For that reason Stakman and Levine prepared a table that gives the variations and constants in the reaction of each differential variety to each physiologic form of P. graminis tritici. Since this table was prepared in 1922, it has been extended so as to include the reactions of all forms discovered subsequent to that time. Several foreign forms not known to occur in North America also are included in this key. These data are shown in table 3.

The identification of the physiologic form or forms is not always so simple as one might be led to think from reading a description of the routine

followed in making an identification. Often there may be 2, 3, or sometimes even more physiologic forms in the same field collection. This necessitates the isolation of each form and the determination of its identity separately from the others. In some cases the forms in a mixture can be identified quite easily. For instance, if there are 2 physiologic forms present that react differently on Marquis, 1 form producing a type-2 infection and the other a type-4, and if there is no evidence of differences on any other variety, no additional inoculations are necessary. The 2 forms may be identified immediately, since it is clear that they are identical in all respects except for the differences on Marquis. The key then will show what these 2 forms are.

A rather simple mixture, yet one which would require some additional inoculations, might consist of 2 forms that differ on Marquis and also in their actions on several durum varieties, 1 form perhaps producing a type-4 infection on these differentials and the other only distinct flecks. It would be clear that there were 2 forms, 1 that attacked Marquis and 1 to which Marquis was resistant; likewise, 1 to which the durum varieties were susceptible and 1 to which they were resistant. But it would not be known whether the form to which Marquis was resistant was producing the weak type of infection or the strong type on the durums. Cross inoculations are then necessary in order to make the correct identifications. There may be far more complex mixtures than those cited above; cultures often have been carried for a year or more before final determinations could be made.

TABLE 1.—List of differential varieties, with their Cereal Investigations numbers and abbreviations, used in identifying physiologic forms of Puccinia graminis tritici

Triticum compactum	
Little Club, C. I. 4066	
Triticum vulgare	
Marquis, C. I. 3641	
Kanred, C. I. 5146	
Kota, C. I. 5878	
Triticum durum	
Arnautka, C. I. 1493	
Mindum, C. I. 5296	
Spelmar, C. I. 6236	
Kubanka, C. I. 2094	
Acme, C. I. 5284	
Triticum monococcum	
Einkorn, C. I. 2433	
Triticum dicoccum	
Vernal, C. I. 3686	
Khapli, C. I. 4013	

TABLE 2.—Analytical key for the identification of physiologic forms of Puccinia graminis tritici on the basis of their parasitic behavior on differential varieties within the genus Triticum

Infection homogeneous	
Little Club resistant	Form 41
Little Club susceptible	
Marquis resistant	
Kanred resistant	
Kota resistant	
Arnautka resistant	
Kubanka resistant	Form 2
Kubanka susceptible	
Einkorn resistant	Form 27
Einkorn susceptible	Form 23
Arnautka susceptible	
Arnautka susceptible Mindum resistant	Form 6
$egin{aligned} ext{Mindum susceptible} \end{aligned}$	
Kubanka resistant	Form 4
Kubanka susceptible	
Einkorn resistant	Form 16
Einkorn susceptible	
Vernal resistant	Form 14
Vernal susceptible	Form 53
Kota susceptible	
Mindum resistant	Form 28
Mindum susceptible	Form 19
Kanred susceptible	
Kota resistant	
Arnautka resistant	
Kubanka resistant	Form 7
Kuhanka sussantihla	
Vernal resistant	Form 33
Vernal susceptible	
Arnautka susceptible	
Kota susceptible	Form 39
Marquis susceptible	1 01111 00
Kanred resistant	
Kota resistant	
Arnautka resistant	
Kubanka resistant	Form 54
Kubanka susceptible	
Arnautka susceptible	I OIM II
Acme resistant	Form 46
Acme susceptible	I OIM TO
Khapli resistant	Form 24
Khapli susceptible	Form 42
	r orm 42
Kota susceptible	
Arnautka resistantVernal resistant	
Vernal susceptible	Form 57
Arnautka susceptible	
Mindum resistant	Form 26
Mindum susceptible	
Kubanka resistant	
Vernal resistant	
Vernal susceptible	Form 8
Kubanka susceptible	
Einkorn resistant	Form 21

TABLE 2—(Continued)

Einkorn susceptible	
Vernal resistant	Form 17
Vernal susceptible	Form 9
Kanred susceptible	
Kota resistant	Form 35
Kota susceptible	
Arnautka resistant	
Mindum resistant	
Kubanka resistant	Form 3
Kubanka susceptible Acme resistant	
	Form 20
Acme susceptible	
Einkorn resistant	
Einkorn susceptible	Form 18
Mindum susceptible	
Spelmar resistant	
Spelmar susceptible	Form 22
Arnautka susceptible	
Mindum resistant	Form 12
Mindum susceptible	
Kubanka resistant	Form 13
Kubanka susceptible	
Einkorn resistant	
Vernal resistant	
Vernal susceptible	Form 40
Einkorn susceptible	
Vernal resistant	
Vernal susceptible Infection heterogeneous Marquis resistant	Form 15
Kanred resistant	
Arnautka resistant	
Arnautka susceptible	Form 45
Arnautka indeterminate	
Acme susceptible	Form 48
Acme indeterminate	Form 47
Kanred susceptible	Form 38
Marquis susceptible	
Kanred resistant	
Kota resistant	
Arnautka resistant	
Arnautka susceptible	Form 55
Kota susceptible	
Arnautka resistant	Form 49
Arnautka susceptible	Form 37
Arnautka indeterminate	
Vernal resistant	Form 29
Vernal susceptible	Form 30
Kanred susceptible	
Kota resistant	Form 31
Kota susceptible	
Arnautka resistant	
Einkorn susceptible	
Vernal resistant	Form 36
Vernal susceptible	Form 52
Arnautka indeterminate	
TITIER ON THE OF THE OWN THE O	

TABLE 3.—Mean infections produced by physiologic forms of Puccinia graminis tritici on differential varieties of Triticum spp.

					Reaction	on of d	ifferent	ial var	ietiesa		1	
Physiologic forms	Little Club	Marquis	Kanred	Kota	Arnautka	Mindum	Spelmar	Kubanka	Acme	Einkorn	Vernal	Khapli
1	$\begin{array}{cccccccccccccccccccccccccccccccccccc$	4-2-4-2-4-2-4-4-2-4-4-4-4-4-4-4-4-4-4-4	0 2= 4= 1-0; 1= 3+0.0 0 3++4= 0 0 0.4= 0; 0 0 0.3++4= 0; 0 0.3++4= 4-0; 0 0.3+4= 4-0; 0 0.3+1+4= 4-0; 0 0.3+1+4= 4-0; 0 0.3+1+4= 4-0; 0 0.3+1+4= 4-0; 0 0.3+	3+ 2= 3+ 2= 3 0; 1= 4- 3++ 2 3+ 3+ 3++ 1++ 3++ 3++ 3++ 3++	1= 1- 1= 4= 4= 4= 4= 4= 4= 4+ 4 4= 4= 1 1 4= 1- 1 1 4= 4- 1 4=	1 1 = + + + + + + + + + + + + + + + + +	1= 1= 1- 3+ + 2= 1- 4= 4	3+ 1+ 1+ 2- 1+ 1- 1- 1- 1- 1- 1- 1- 1- 1- 1-	3++ 3++ 3++ 3++ 3++ 3++ 3++ 3++	3 3+ 3+ 3+ 3+ 3 3- 3+ 3 3+ 3+ 3 3- 3+ 3 3- 3+ 3 3- 3+ 3 3- 3+ 3 3- 3 3-	0; 1- 1= 0; 0. 1 4= 1= 1= 1- 0; 1- 1= 1- 0; 1- 1- 1- 1- 1- 1- 1- 1- 1- 1-	1= 0; 0; 1= 0 0; 1= 0; 1= 0; 1= 1= 0; 1= 1= 1= 1- 1= 1 1- 1= 1+ 1+ 1- 1= 1+ 1- 1= 1+ 1- 1= 1+ 1+ 1- 1= 1+ 1+ 1- 1= 1+ 1+ 1- 1= 1+ 1+ 1- 1= 1+ 1+ 1- 1= 1+ 1+ 1- 1= 1+ 1+ 1- 1= 1+ 1+ 1- 1= 1+ 1+ 1- 1= 1+ 1+ 1- 1= 1+ 1+ 1- 1= 1+ 1+ 1- 1= 1+ 1+ 1- 1= 1+ 1+ 1- 1= 1+ 1+ 1- 1= 1+ 1+ 1- 1= 1+ 1+ 1- 1= 1+ 1+ 1- 1= 1+ 1+ 1+ 1- 1= 1+ 1+ 1+ 1- 1= 1+ 1+ 1+ 1+ 1+ 1+ 1+ 1+ 1+ 1+ 1+ 1+ 1+

TABLE 3—(Continued)

				Rea	action o	f diffe	rential	varietie	Sa			
Physiologic forms	Little Club	Marquis	Kanred	Kota	Arnautka	Mindum	Spelmar	Kubanka	Acme	Einkorn	Vernal	Khapli
50	4+ 4 4 4 4 4 4 4	2+ 2= 4 2± 3++ 4 3+ 4-	0; 3+ 4- 0 0 0 3+ 0.	2++ 0; 4 1 0; 2- 3+ 3+	1- 0; 1= 4 0; 4 1= 1	0; 0; 1= 4 0; 4 1= 1	0; 0; 1= 4 0; 4 1= 1	x= 4 x+ 4 1 x 3+ 4	x= 3++ 4 4 3+ x 3+ 3+	0; 3+ 4- 3± 3 3 1= 3	0; 4- 4+ 3± 1 3 1= 3	0; 0; 1- 1 0; 1- 1-

a Explanation of symbols: 0—absolute immunity, 1—extreme resistance, 2—moderate resistance, 3—moderate susceptibility, 4—complete susceptibility, x—indeterminate reaction, (;)—hypersensitive flecks, (.)—necrotic lesions, (:)—hypersensitive islands; plus and minus signs indicate a slightly greater or smaller amount of rust than the nearest figure representing the infection types; c—chlorosis, n—necrosis.

RESULTS OF THE PHYSIOLOGICAL-FORM SURVEY IN 1926

Physiologic forms isolated: their prevalence and distribution. In 1922 Stakman and Levine (13) described 37 physiologic forms of Puccinia graminis tritici. All of these had been found at one time or another in North America. Between 1922 and 1926 2 additional forms were identified from rust collections made in the United States, thus making a total of 39 forms known to exist in North America. In 1926, 1927, and 1928 8 additional forms were discovered in the United States and Canada.

The following 18 forms were isolated in 1926: 1, 3, 11, 14, 17, 18, 19, 21, 23, 29, 30, 32, 33, 34, 36, 38, 39, and 51. Of these, forms 18 and 36 were by far the most common. Together they comprise 65 of the 138 identifications, having been collected 37 and 38 times, respectively. Other forms, such as 30 and 19, were collected only once and twice, respectively.

The distribution and prevalence of the forms isolated by the writer in 1926 are shown in table 4 and in figure 1. They are briefly summarized as follows:

Form 1. Found twice in 1926. Collected in Nova Scotia and Ontario, Canada. Not collected in the United States.

Form 3. Found 9 times. Collected in Colorado, Montana, Ohio, and Oklahoma. Found also in Quebec and New Brunswick, Canada.

Form. 11. Most prevalent form collected in southern United States. Found 6 times in Texas, and once each in Arizona, Georgia, and Kentucky.

Form 14. Collected once in Utah and once in Ontario.

Form 17. Isolated from 9 collections. Widely distributed; found in California, Kentucky, North Carolina, Oklahoma, Texas, Utah, Quebec, and Nova Scotia.

TABLE 4.—Prevalence and distribution of physiologic forms of Puccinia graminis tritici isolated in North America in 1926

							Ι	Phys	siol	ogic	for	ms							Totaln	umbon
Distribution	1	3	11	14	17	18	19	21	23	29	30	32	33	34	36	38	39	51	Totalii	umber
					:	N	Tum	ber	of	tim	es is	ola	ted						Cultures	Forms
United States Alabama Arizona California Colorado Georgia Indiana Ilowa Kansas Kentucky Michigan Minnesota Missouri Montana Nebraska North Carolina North Carolina Ohio Oklahoma Pennsylvania South Dakota Texas Utah Virginia Wisconsin Wyoming		ī	ī1 ī1		1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	1 2 2 2 1 1 2 2 1 1 2 2 3				1			1 1 1 1	i			 4	1	1 3 1 5 1 3 2 5 4 4 2 2 1 4 8 1 6 5 2 1 7 6 1 7 6 1 7 6 1 7 6 1 7 6 1 7 6 1 7 6 1 7 6 1 7 6 1 7 6 1 7 6 1 7 6 1 7 7 6 1 7 7 8 7 8 7 8 7 8 7 8 7 8 7 8 7 8 7 8	1314121443813313421376132
Canada Quebec New Brunswick Nova Scotia Ontario	1	3 . 1 		 ï	1	1				1		3 2			3 1				13 2 3 4	6 2 3 3
Number of times each form was found	2	9	9	2	9	37	2	12	2	2	1	11	1	1	28	2	7	1	138	18

Form 18. The most prevalent form in 1926. It was wide-spread; found in Utah, Arizona, Texas, and Nova Scotia, as well as rather generally distributed over intermediate areas. Isolated 37 times during the season.

Form 19. Found twice. Collected in Texas and Quebec.

Form 21. Third in prevalence among the forms isolated. Found 12 times; quite generally distributed. Collected in Colorado, Kansas, Kentucky, Michigan, Minnesota, Nebraska, North Dakota, and Texas.

Form 23. Found only twice during the season. Collected in Pennsylvania and Virginia.

Form 29. Found once in Indiana and once in Quebec.

Form 30. One collection of form 30, late in the season at Fort Duchesne, Utah.

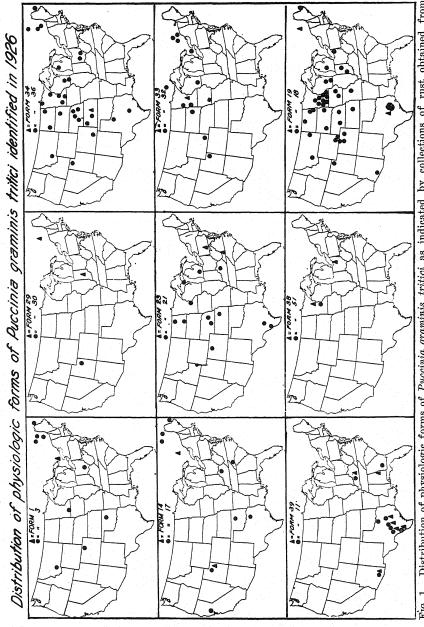


Fig. 1. Distribution of physiologic forms of Puccinia graminis tritici as indicated by collections of rust obtained from different States in the United States and from Canada and identified in 1926,

Form 32. Isolated from 11 collections. Found in Minnesota, South Dakota, Utah, Wisconsin, and Wyoming, in the United States, and in Ontario and Quebec, in Canada.

Form 33. Collected once in Minnesota.

Form 34. Found once in Kansas.

Form 36. Found 28 times; distributed over practically the same area as form 18. Collected in 12 States of the United States and in 2 Provinces of Canada.

Form 38. Found twice, once in Minnesota and once in Ohio.

Form 39. Apparently restricted to the South. Distribution much the same as that of form 11. Collected 4 times in Texas and once, each, in Arizona, Alabama, and Kentucky.

Form 51. Collected once in St. Paul, Minn.

Identity and nature of isolated physiologic forms. The reactions of the differential varieties in the greenhouse to the forms isolated in 1926 are as follows. Of the *vulgare* wheats Marquis was susceptible to 11 of the 18 forms. Kanred was susceptible to 10, highly resistant to 4, and immune from the remaining 4 forms. Kota was susceptible to 14 of the 18 forms.

Of the durum wheats, Arnautka, Mindum, and Spelmar reacted the same. They were susceptible to 7 forms, resistant to 7, and reacted heterogeneously 5 to 4 forms. Kubanka was susceptible to 12 forms and resistant to 1 and gave a heterogeneous reaction to 5. Acme was susceptible to 14 forms and reacted heterogeneously to 4.

Little Club, the only *compactum* wheat in the list of differentials, was susceptible to all forms isolated, while Einkorn, C. I. 2433, the only representative of *Triticum monococcum*, was susceptible to 17 of the 18 forms.

Of the emmers, Khapli was resistant to all of the 18 forms and Vernal was resistant to all but 2.

Evidence of migration of rust from South to North: (Comparison of southern and northern physiologic forms.) A comparative study of the southern and northern physiologic forms in 1926 suggests that there was no general migration of stem rust from South to North.

This year form 11 was the most common form found in Texas. It was found also in Arizona, Georgia, and Kentucky but was not identified in the Minnesota laboratory from any other States. Six of the 17 collections from Texas were form 11. Although this form was not identified by the writer from either the hard-red-winter or hard-red-spring-wheat regions, Peltier and Thiel (11) report having collected it 13 times in 1926. The majority of these 13 collections were made in Nebraska, but these authors mention that they identified form 11 also from collections made in Arizona and

⁵ On a single leaf there may be a range of infection from necrotic flecks to type-4 pustules not subject to mechanical separation.

Texas. They found form 11 on wild grasses near infected barberries and also in grain fields where the infection could not be traced to barberries. Peltier and Thiel (11) are of the opinion that form 11 originated on barberries in northeastern Nebraska and that possibly some inoculum of this form may have come from other sources out of the State.

Stakman, Levine, and Wallace (16) suggest that Peltier and Thiel may have, in some instances at least, identified form 32 as form 11. Form 32 is identical with form 11 except that it produces a heterogeneous or type-x infection on the durum varieties, whereas form 11 produces a type-4 infection. Under certain environmental conditions the type-x infection may appear as type-4, thus causing the collection to be identified as form 11. It can not be said with certainty that this actually occurred in the case of the collections of Peltier and Thiel, but that such may have happened is suggested by the statement of these authors regarding the differences in degree of virulence of different collections of form 11. They found collections of this form to be outstandingly different in virulence. To the writer, this suggests that Peltier and Thiel were dealing with a form that produces the heterogeneous, or type-x, infection on the durum varieties, very likely form 32.

Form 39 seemed rather closely associated in 1926 with form 11, as these 2 forms were found together in 4 locations in Texas and also at Sacaton, Ariz., and Lexington, Ky. It was found also in Alabama but was not collected in the States north of Texas.

Form 186 was found in Texas, at College Station, and form 36 was found at Georgetown and Chillicothe, Tex. These forms were the 2 most common in the northern collections. Form 21 was found twice in Texas and was found later in 10 other collections from Nebraska, Kansas, Kentucky, Colorado, North Dakota, Minnesota, and Michigan. Since forms 18, 21, and 36 were found to be those most common in collections from the northern wheat fields, and, since all 3 of them were known to be present in Texas, it might seem that these forms may have migrated from Texas. However, further study of the prevalence and distribution of forms other

6 Some doubt has recently developed as to the differentiation between form 18 and form 36. Their behavior is identical on all differential varieties with the exception of Kubanka. Form 18 produces a 4-type infection on Kubanka, indicating complete susceptibility. Form 36 produces the heterogeneous, or type-x, infection. Under certain environmental conditions form 36 may produce what appears to be a type-4 infection, the flecks and small pustules of the type-x apparently being masked by the large pustules. In such instances, if additional inoculations are not made on Kubanka, the culture might be identified as form 18. It is believed that form 18 actually exists, yet it is interesting to know that at the Dominion Rust Research Laboratory of Canada form 18 was not identified at all between 1923 and 1928, while over 500 collections were identified as form 36. In this paper forms 18 and 36 are discussed as 2 distinct forms.

than 18, 21, and 36 does not support the assumption that there was a general migration of stem rust from the South to the North.

Since forms 11 and 39 were more prevalent in Texas wheat fields than other forms and were not found elsewhere in the central wheat region, except possibly form 11 in Nebraska, it is difficult to explain how forms 18, 21, and 36 could have been blown northward from Texas, leaving forms 11 and 39 behind. This, likewise, applies to form 19, which was found in Texas but at no other place in the United States.

There is additional evidence that form 21 did not migrate northward from Texas. Forms 21 and 11, respectively, were collected in the same field at Laredo, Tex. At San Antonio form 21 was collected, and form 11 was found at Castroville, only a few miles from San Antonio. If form 21 migrated northward from these locations in Texas it seems reasonable to assume that form 11 also would have migrated because it has a wider host range than form 21. It attacks Kanred wheat, which is immune from form 21, and considerable Kanred wheat is grown in the hard-red-winter-wheat region.

Form 17 was collected at Denton, in northern Texas, and form 11 was isolated from the same collection. Form 17 was collected later at Stillwater, Okla., along with form 3. Form 11 was not found in Oklahoma and form 3 was not found at all in Texas. However, form 11 might have been present in Oklahoma, as only 1 rust collection was identified from that State. Apparently, form 17 did not spread from either Texas or Oklahoma to the more northern regions of the Mississippi Valley. It was found in North Carolina, Utah, California, Kentucky, and eastern Canada, Oklahoma, and Texas, but not elsewhere.

Form 32 ranked fourth in prevalence in 1926. It was collected 10 times and was present in Utah, Wyoming, South Dakota, Minnesota, and Wisconsin, and in Canada. This form was not collected in the southern United States.

Since a rather severe epiphytotic of stem rust occurred in Arizona and southern California in 1926, this area naturally has been considered as a possible source of inoculum.

Only 1 collection of stem rust was obtained from Arizona, from which were isolated forms 11, 18, and 39. Nine collections were made in north-eastern Colorado and southeastern Wyoming. Five of these were form 18. Forms 11 and 39 were not collected in Colorado or Wyoming.

Comparison of United States survey with Canadian survey. Data on the survey conducted in 1926 by the Dominion Rust Research Laboratory at Winnipeg, have recently become available (7, 8) and it is of interest to note these results. The results of the Canadian survey are compared with those of the United States in table 5. Fifteen forms were isolated by the Canadian workers in 1926. Ten of these were found by the writer in 1926. The other 5 forms, isolated in the Canadian survey and not found by the writer, comprise collectively only a total of 9 collections. Three of these forms, namely, 48, 49, and 52, had never been identified before. The writer isolated 8 forms that were not identified in the Canadian laboratory. These were 1, 3, 11, 18, 23, 33, 39, and 51. Forms 1 and 3 were isolated from Canadian collections. Of the 13 forms not isolated by both laboratories, probably only 3 were common enough to be of any significance in studying rust migration. Form 3 was not found in the Canadian survey, but it occurred in Canada, since the writer isolated it from 4 rust collections from Quebec and New Brunswick. It is interesting to note that forms 11 and 39 were not identified from any

TABLE 5.—Summary of the prevalence of physiologic forms of Puccinia graminis tritici in 1926 as shown by the surveys of the United States Department of Agriculture and of the Dominion Rust Research Laboratory of Canada

Physiologic	Nu	mber of times isola	ated
forms	United States	Canada	Both surveys
1	2		2
3	9		9
9	•••	1	1
11	9		9
14	2	2	4
15	••••••••••••••••••••••••••••••••••••••	2	2
17	9	2	11
18	37		37
19	2	1	3
21	12	86	98
23	2		2
29	2	29	31
30	1	7	8
32	11	12	23
33	1		1
34	1	4	5
36	28	217	245
38	2	18	20
39	7		7
48		2	2
49		3	3
51	1		1
52		1	1
Total number of isolations	138	387	525
Total number of forms			* 1
isolated	18	15	23

Canadian collections. It seems safe to conclude that had forms 11 and 39 migrated northward from Texas they would have been isolated at least a few times from the approximately 500 collections made in the northern United States and Canada.

In Canada form 36 was most prevalent by far, but it should be remembered that all collections of form 18, if there is such a form, are listed by the Canadian workers as form 36. Form 21 ranked second in Canada and this is in agreement with the United States survey. Form 29 ranked third and this form was found only once in the United States. It appears that form 29 certainly must have originated on barberries. Whether these were local barberries or not is undetermined.

PHYSIOLOGICAL FORMS EXPECTED FROM BARBERRIES IN 1926

If it were possible to collect and identify a large number of aecial infections on barberries each year and then correlate the physiologic forms on barberries with the forms occurring in the fields, both in the barberry region and in the South, the source of inoculum could be rather definitely established. For instance, if the predominant forms found on barberries are the same as those occurring on wheat and grasses in the spring-wheat area but different from the southern forms, then it would be quite certain that the barberries were responsible for the stem rust occurring that year. In a similar way it could be shown that both the barberries and the southern wind-blown inoculum contributed towards an epiphytotic or that only the southern source was of importance.

Since the 1926 survey was well advanced before the writer outlined this particular problem, no aecial collections were obtained. In 1927 and 1928 an attempt was made to obtain aecial material and to make identifications of such collections. The results were quite disappointing, however, as no satisfactory method of obtaining fresh, viable aecia was devised. In 1928, over 200 aecial collections were obtained, but relatively few produced infection after being transferred to the proper hosts.

It would be expected that physiologic forms common one year would be likewise common the next year through the agency of barberries, but with the many factors, such as teliospore viability and germination, infection of barberries, and recent evidence of the origin of new forms through hybridization (10, 19) influencing the behavior of stem rust, there may be no regular correlation between the occurrence of certain forms one year and their occurrence the following year. The writer, as well as others, has noticed the extreme scarcity or even absence of collections of certain forms in one season even though these same forms were quite common the previous season. That certain forms have been rather regular in their occurrence

⁷ Stakman and Levine-unpublished data.

and then apparently disappear for 1, 2, or more years, is a well-established fact, as far as the annual surveys demonstrate, but an explanation for such behavior is still lacking. More extensive surveys with more carefully planned field collections might reveal the presence of every known form each year. However, it seems that the collections, though limited in number, should be indicative of the relative prevalence of those forms to which the commonly grown wheat varieties are susceptible.

The 4 most commonly isolated forms in the United States in 1926 were 18, 36, 21, and 32. The survey records show that forms 18, 32, 21, and 11 were the most prevalent in 1925. The scarcity of form 36 in 1925 could possibly be explained by the fact that many collections may have been identified as form 18 that in reality were form 36. Form 11 ranked fourth in 1925 but was collected only 9 times and that over a wide area.

In 1925, in Canada, only 3 forms were of very much importance, according to the survey conducted at the Dominion Rust Research Laboratory. They were, in order of their prevalence, forms 36, 21, and 29. In 1926 these three forms again predominated and their increased numbers of isolations in 1926 were almost exactly proportional to the increase of the total collections of 1926 over those of 1925. In 1926 form 38 ranked fourth in This form had never been identified there previously. Canada. probably explained by the fact that until 1926 the Canadian survey included only the western Provinces, i.e., Manitoba, Saskatchewan, and Al-In 1926 the eastern Provinces were included in the survey and form 38 was collected in that region 17 times, whereas it was collected only once in the western Provinces. Newton, Johnson, and Brown (9) describe form 38 as characteristically an eastern form. The writer finds this to be somewhat the case as shown by surveys conducted at the University of Minnesota. Since Marquis is resistant to form 38, Newton, Johnson, and Brown attribute its prevalence in eastern Canada to the growing of many different wheat varieties, most of which are susceptible to form 38. They conclude that its gradual spread westward has been influenced by the introduction of susceptible varieties. Mention has been made of form 38 here because of the fact that it was again rather common in 1927, as will be brought out later in this paper.

The rather common occurrence, in 1926, of certain physiologic forms that had been the most common forms in 1925, as shown by both the United States and Canadian surveys, suggests that the northern rust in 1926, probably, for the greater part, originated on barberrries.

RESULTS OF THE PHYSIOLOGIC-FORM SURVEY IN 1927

Physiologic forms isolated: their prevalence and distribution. In 1927, the following 19 physiologic forms were isolated: 1, 3, 11, 14, 17, 18, 19.

21, 23, 28, 30, 32, 33, 36, 38, 39, 49, 50, and 52. Form 52 had never before been isolated in the United States, but it had been found in 1926 by the Canadian workers at the Dominion Rust Research Laboratory, at Winnipeg. Peltier and Thiel (11) collected a new form 4 times in Nebraska in 1926, which was later given the number 49. Four hundred and ten identifications were made in 1927. As in 1926, form 18 was the most prevalent of the forms identified and form 21 was a close second. Forms 39 and 36 ranked third and fourth, respectively.

The prevalence and distribution of those forms isolated in 1927 are given in table 7 and figure 2 and are summarized as follows:

Form 1. Isolated 8 times; found in Texas, Oklahoma, Kansas, Illinois, Idaho, Wisconsin, and Pennsylvania.

Form 3. Found once in Montana.

Form 11. Isolated from 17 collections; found in Texas 7 times, Kansas 3 times, Ohio twice, and once, each, in Tennessee, North Dakota, Missouri, Indiana, and Iowa.

Form 14. Found once in Montana and twice in California.

Form 17. Isolated from 17 collections. Found in Texas, Oklahoma, Nebraska, South Dakota, Missouri, Minnesota, Montana, and Pennsylvania.

Form 18. Extremely common and wide-spread. Found in 21 of the 25 States included in the survey. Isolated from 124 collections.

Form 19. Found 8 times. Twice, each, in Texas and Kansas and once, each, in Minnesota, Montana, Nebraska, and Pennsylvania.

Form 21. Found 120 times; widely distributed, collected in 21 States. Its prevalence and distribution were essentially the same as those of form 18.

Form 23. Found twice only, both collections from Pennsylvania.

Form 28. Isolated once in Texas; not found elsewhere.

Form 30. Collected once in Illinois.

Form 32. Found 14 times; quite widely distributed.

Form 33. Found once, each, in Wisconsin, Michigan, and Ohio.

Form 36. Isolated 29 times from collections in 14 different States.

Form 38. Collected 18 times from 10 States. Rather common in collections from Eastern States.

Form 39. This form ranked third in prevalence, being isolated 33 times; rather generally distributed; collections made in 15 States.

Form 49. Although form 49 had never been found in the United States previous to 1927, except in Nebraska, it was collected 9 times in the United States in 1927, from 8 States.

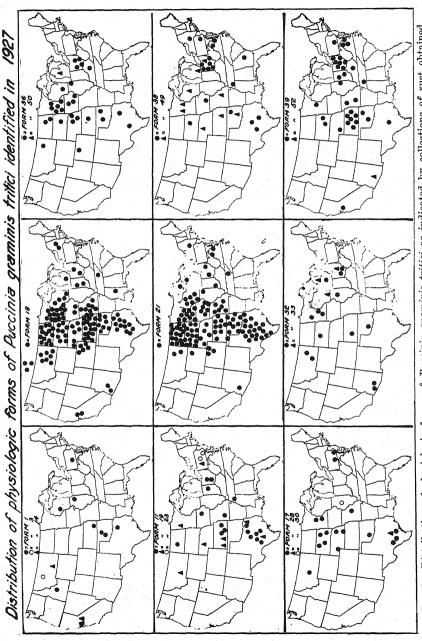
Form 50. Collected for the first time in the United States in Michigan.

Form 52. Found for the first time in the United States; collected only once, in Arizona. This form was found once in 1926 by the Canadian investigators.

TABLE 6.—Prevalence and distribution of physiologic forms of Puccinia graminis tritici isolated in North America in 1927

	Physiologic forms	number
	1 3 11 14 17 18 19 21 23 28 30 32 33 36 38 39 49 50 52	rumber
Distribution	Number of times isolated Cultures	Form
United States Arizona California Colorado Georgia Idaho Illinois Indiana Iowa Kansas Kentucky Michigan Minnesota Mississippi Missouri Montana Nebraska New York North Dakota Ohio Oklahoma Pennsylvania South Dakota Tennessee Texas Virginia Wisconsin Wyoming Canada Saskatchewan	$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	4 3 3 1 1 6 4 5 9 2 6 8 1 6 8 7 3 4 9 9 6 7 5 1 0 4 9 6 7 5 1 0 4 9 6 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2
Number of times each form was found	8 1 17 3 17 124 8 120 2 1 1 14 3 29 18 33 9 1 1 410	19

Identity and nature of isolated physiologic forms. The reactions of the 12 differential varieties to each of the physiologic forms isolated in 1927 are as follows. Little Club was susceptible to all the 1927 forms. Marquis was resistant to 8, Kanred to 10, and Kota to 4 of the 19 forms isolated. The durum varieties, Arnautka, Mindum, and Spelmar, reacting as a group, were resistant to 9 of the forms and responded heterogeneously to 3 others, producing the type-x infection. One form was collected to which Mindum was resistant, while all other durum varieties were susceptible. Kubanka was resistant to only 1 form and reacted heterogeneously to 7 forms. Acme was susceptible to 15 forms and reacted heterogeneously to the other 4.



Distribution of physiologic forms of Puccinia graminis tritici as indicated by collections of rust obtained from different States in the United States and from Canada and identified in 1927. Fig. 2.

Einkorn was resistant to 3 forms. Only 2 forms were found that attacked Vernal heavily, namely, forms 30 and 52, each of which was found only once. None of the 19 forms isolated was capable of producing more than flecks and very minute type-1 pustules on Khapli.

Evidence of migration of rust from south to north: Comparison of southern and northern physiologic forms. In 1927, 410 identifications were made by the writer from rust collections in North America. Of these, 406 were made from rust collected in the United States. The places of collection were more or less uniformly distributed, especially over the principal wheat-growing States of the Mississippi Valley. Fifty-two identifications were made from Texas collections, 28 from Oklahoma, 43 from Kansas, 20 from Nebraska, 18 from Missouri, and the hard-red-spring-wheat States were, likewise, fairly well covered. More collections than were obtained from the Great Lakes States would have been desirable in order to more accurately compare that region with Texas as a source of inoculum for the hard-red-spring-wheat States. However, there were 60 identifications made from Wisconsin, Illinois, Indiana, Michigan, and Ohio collections, as compared to 52 identifications from Texas collections. If aeciospores produced on barberry bushes in the Great Lakes States were responsible for any of the rust that developed later in the wheat fields of Minnesota and the Dakotas, these same barberry bushes would be expected to be responsible for a considerable part of the field infection in the Great Lakes States. Even though the numbers were relatively small, it seems that random field collections would yield the forms that had become best established in a particular region. Forms to which most of the commonly grown wheat varieties are resistant naturally stand less chance of being collected.

The larger number of identified collections in 1927 over those of 1926 gave much better data for studying the possible migration of rust from South to North. From the available data, it does not appear to the writer that the Great Lakes States were so important as a source of inoculum in 1927 as was Texas. There was a total of 72 identifications made from Illinois, Indiana, Michigan, Ohio, Pennsylvania, New York, and Wisconsin. Thirty-three per cent of these were forms 18 and 21, whereas these 2 forms comprised a total of 73 per cent of the 52 identifications from Texas. Wisconsin is so located geographically that it could have received inoculum from both sources, so that it may be fairer to omit it from the Great Lakes States group. Without the Wisconsin collections forms 18 and 21 would comprise 24 per cent of the total of 58 identifications from the other Great Lakes States.

It is of further interest to note that in some of the Great Lakes States there was no outstanding form, so far as the collections showed. Six identifications from Illinois produced 6 different forms; 4 from Indiana, 3 forms; 7 from Michigan, 6 forms; 4 from New York, 3 forms; 8 from Penn-

sylvania, 6 forms. Ohio was an exception to this, as there were 9 forms from 27 identifications, forms 36, 38, and 39 being collected more often than any others. In Minnesota there were 8 forms in 40 identifications; in North Dakota there were only 4 forms in 33 identifications; and in South Dakota 7 forms were found in 32 identifications. In a total of 105 identifications from the last 3 named States there were only 10 forms. Fifteen forms were isolated from 72 cultures of rust from the Great Lakes States.

The above data suggested that in the barberry area there may occur a greater variety of forms than in more distant areas. Theoretically, this should be expected.

Form 38 was found 6 times in Ohio and form 39 was found 7 times. Form 38 was found once in New York and once in Pennsylvania, while form 39 was found once in Illinois, once in Pennsylvania, and twice in Indiana. Both forms 38 and 39 were rather scarce in the collections from the hard-red-spring-wheat States, being found only once and 3 times, respectively, in the whole of Wisconsin, Minnesota, North Dakota, South Dakota, and Montana.

Forms 1, 23, 30, 33, and 50 were collected in the Great Lakes States in 1927 but were not recovered from any of the collections in Minnesota, North Dakota, South Dakota, and Montana. Marquis and some of the durum wheats are resistant to forms 23, 33, and 50, but forms 1 and 30 should have the same chance of finding susceptible varieties as forms 18 and 21.

In 1927 forms 18 and 21 were predominant, having been collected 124 and 120 times, respectively. Their prevalence and regular distribution are quite striking, as shown by the maps in figure 2. Beginning with Texas, where these 2 forms were found 12 and 20 times, respectively, and, moving northward, they were collected as follows: Oklahoma, 8 and 10; Kansas, 18 and 9; Nebraska, 6 and 5; Missouri, 7 and 6; South Dakota, 9 and 14; North Dakota, 14 and 16; Montana, 7 and 2; Minnesota, 15 and 10; and Wisconsin, 4 and 6. The regularity with which these 2 forms were collected throughout the season, after having been outstandingly common in Texas in the spring, suggest a possible dissemination of inoculum from Texas to the Northern States.

Additional evidence that there was an appreciable migration of stem rust from the south in 1927 lies in the fact that of the 10 most prevalent forms over the entire surveyed area 9 were present in Texas. Form 32 was found 14 times but was not collected in Texas. Form 28 was collected once in Texas but not elsewhere.⁸ Of the 19 forms isolated in 1927, only 10 forms were found more than 3 times, each. With the exception of form 32, all physiologic forms found more than 3 times, each, during the year also were collected in the same season in Texas.

s Marquis, Mindum, and Kanred varieties of wheat are highly resistant to this form. This may decrease the possibility of collecting it in the hard-red-spring- and durum-wheat fields. It never has been a common form, so far as the surveys indicate.

COMPARISON OF UNITED STATES SURVEY WITH CANADIAN SURVEY

A summary of the results obtained by the Dominion Rust Research Laboratory survey of 1927 and of the United States Department of Agriculture survey of the same year is given in table 7. At Winnipeg 511 identifications were made and 19 physiologic forms were isolated. The 2 most prevalent forms in the United States in 1927 were 18 and 21, whereas in Canada they were forms 36 and 21. It should be remembered that at the Canadian laboratory form 18 is not distinguished from form 36. If forms 18 and 36 were combined in the United States as is done in Canada, there would have been 153 collections of form 36. Then, with 120 collections of form 21, forms 21 and 36 would comprise 273 of the 410 identifications made, or about 67 per cent. In Canada these 2 forms comprised 369 of the 511 identifications, or approximately 72 per cent. These data certainly seem to indicate that most of the stem rust of wheat in Canada in 1927 originally came from the same source as that in the Mississippi Valley of the United States. It seems also to support the belief that in 1927 there was a migration of stem rust of wheat from South to North. It is quite clear that the southern wheat fields are not the only source of inoculum for Canadian fields It is the writer's contention that under favorable climatic conditions the barberry is always responsible for a certain amount of stem rust but that severe and widespread epiphytotics probably result only when both sources supply inoculum and when proper environmental factors are present for the development and dissemination of urediniospores. That western Canada receives inoculum from other sources than the adjoining States of the United States is brought out by the fact that each year certain forms that are not found at all in the United States occur rather commonly in Canada. In the Prairie Provinces, Manitoba, Saskatchewan, and Alberta, form 15 was collected 9 times in 1927. This form was not collected in the United States a single time. In this same region form 14 was collected 15 times. It was collected only once in the United States, in Utah. Likewise, forms 16, 29, 34, 56, and 27 were collected 2, 5, 12, 1, and 1 times, respectively, in western Canada and were not found in the United States at all. Form 52 was collected 13 times in Manitoba and Saskatchewan and was found only once in the United States (Arizona), the only time it has ever yet been found in the United States. It would seem that the lateness of the season in Canada would permit the accumulation of rust forms from many different regions.

Physiologic forms of stem rust of wheat expected from barberries in 1927. Since the eastern United States was not covered sufficiently by the survey of 1926, it is hardly worth while to speculate as to the forms that might be expected on barberries there in 1927. In the Middle West, the only forms that would be expected in 1927, because of their abundance in

TABLE 7.—Summary of the prevalence of physiologic forms of Puccinia graminis tritici in 1927 as shown by the surveys of the United States Department of Agriculture and of the Dominion Rust Research Laboratory of Canada

Physiologic	Nu	mber of times isola	ted
forms	United States	Canada	Both surveys
1	8		8
3	1		1
9		7	7
11	17	******	17
14	3	16	19
15		9	9
16		2	2
17	17	18	35
18	124		124
19	8	1	9
21	120	141	261
23	2		2
28	1	•••••	1
29		5	5
30	1	10	11
32	14	7	21
33	3		3
34		12	12
36	29	228	257
38	18	25	43
39	33	<u></u>	33
49	9	10	19
50	1	4	5
52	1	13	14
53	<u></u>	1	1
56		1	1
57		1	1
Fotal number of			
isolations	410	511	921
Total number of			
forms isolated	19	19	27

1926, were forms 18, 21, 36, and possibly 32. The first 3 forms were very common in 1927 and no doubt these forms were disseminated to some extent by barberries. However, as discussed previously, it appears that part of the damage done by these 3 forms in 1927 was due to their abundance in southern fields early in the season and their consequent migration northward. Form 32 evidently did not come from Texas in 1927, and it was not found there at all. Apparently, it came principally from barberries, although it was obtained once from the 28 collections made in Oklahoma.

It was not common anywhere, being found only 14 times, a small number as compared with the collections of forms 18 and 21.

On the other hand, certain forms were common in 1927 in the barberry area of the northern United States that were not found there in sufficient quantity in 1926 to lead one to expect them to occur on barberries in 1927. Forms 11 and 39 were not found at all in the Mississippi Valley north of Texas in 1926. These 2 forms were found 10 and 32 times, respectively, outside of Texas in 1927. Form 11 could easily have migrated from Texas, since it was collected there 7 times. Form 39 was found only once in Texas, twice in Oklahoma, and 7 times in Kansas. That spores of this form developed in Texas and migrated northward, increasing in number on the way, is possible; but, since it was rather common in Ohio and was found also in Virginia, Kentucky, Pennsylvania, Indiana, and Illinois of the States east of the Mississippi River, it is possible that it may have come principally from eastern barberries.

Form 17 was not found in the Mississippi Valley north of Oklahoma in 1926 and therefore it was not expected from barberries in the spring-wheat area in 1927. This form was found 3 times in Texas and 11 times in the other valley States in 1927. This would suggest Texas as its starting place.

The Canadian survey of 1926 showed forms 21, 29, 32, 36, and 38 to be present in sufficient amounts to make it reasonable to expect their recurrence the following year. Forms 29, 32, and 38 were not nearly so prevalent in 1926 as forms 21 and 36, since they were collected only 29, 12, and 18 times, respectively, as compared to 86 collections of form 21 and 217 collections of form 36. In 1927 forms 21 and 36 were collected 141 and 228 times, whereas forms 29, 32, and 38 were collected 5, 7, and 25 times, respectively. Form 29 probably did not migrate into Canada from the United States in 1927, because it was not found at all that year in the States. Form 32 was present in the States but did not seem to be very abundant in any particular locality. Form 38 was somewhat more prevalent in the States and was fairly common in Kansas and Ohio. It is probably unsafe to conclude that these last 3 forms migrated from the United States into Canada, even though the increase in number of collections of form 38 in 1927 over the number of collections of this form in 1926 in the United States and a corresponding increase in Canada suggest a migration of this form during the season. This evidence is strengthened somewhat by the fact that in 1926 form 38 was collected only once in the 3 western Provinces of Canada and was collected 12 times in these Provinces in 1927.

That certain forms evidently must persist from year to year on barberries in Canada is suggested by the results of the Canadian and United States surveys (7, 8, 9, and unpublished data of Stakman and Levine). Mention is here made of some of the evidence supporting this theory, and a more careful study of the above data would no doubt bring out more of such evidence. From 1918 to 1927 form 30 was collected in the United States only 9 times and has been found but twice since 1922. The Canadian survey shows that form 30 was collected 7 times in 1926 and 10 times in 1927. Over this same period of 9 years form 34 has been identified at the St. Paul laboratory only 5 times from collections made in the United States. In Canada form 34 was collected 4 times in 1926 and 12 times in 1927. Why these forms are not better established in the United States is not known. A study of such forms might possibly bring out some interesting physiologic differences, such as response to environmental factors, etc., which might explain their rather capricious behavior.

RESULTS OF THE PHYSIOLOGIC-FORM SURVEY IN 1928

Physiologic forms isolated: their prevalence and distribution. In 1928, 401 identifications were made from collections of stem rust of wheat. An attempt was made to have at least 1 collection from every county in each State. The Mississippi Valley States were covered very thoroughly, but, owing to the hot weather during the summer months, the identification of physiologic forms in the greenhouse was considerably retarded and it was impossible to study all collections that were selected for identification. Approximately 400 collections were placed in cold storage in the hope that the spores would remain viable until inoculations could be made, but no infection was obtained from most of the stored collections when inoculations were made in October and November.

The 1928 survey included 61 identifications of rust collections made in Mexico. No collections had been obtained from Mexico since 1923.

Nineteen different physiologic forms were isolated in 1928. Their prevalence and distribution by States are shown in table 8 and figure 3. In 1928 form 38 was collected most frequently, being identified 105 times. Form 36 was a close second; it was collected 96 times. Forms 21 and 49 ranked third and fourth, being collected 67 and 29 times, respectively. The summary of the prevalence and distribution of the forms is given below.

Form 1. Collected 8 times. Not found farther south than Kansas.

Form 2. Collected once in Minnesota and once in Wisconsin.

Form 11. Found 9 times during the season; once in Mexico, twice in Texas, once in Michigan, and 5 times in Kansas.

Form 17. Collected 14 times. Five of these collections came from Mexico. The remaining 9 collections were made in several of the central and northern Mississippi Valley States and in Arizona.

Form 18. Form 18 was studied carefully during this survey and any collections showing the least tendency towards a heterogeneous, or type-x infection on Kubanka were identified as form 36. Therefore, only 9 collec-

⁹ Collections made by Mr. H. H. Thornberry, who was detailed by the U. S. Dept. of Agriculture to study rust conditions in Mexico in 1928.

tions were identified as form 18. However, these 9 collections were not continued long enough to determine whether they would constantly react as form 18. It is entirely possible that had they been cultured repeatedly they eventually would have produced type-x infection on Kubanka, which would place them with form 36. The 1928 data, obtained through a close observation of the collections of these 2 very similar forms, suggest that many of the collections identified in previous years as form 18 might have been in reality form 36. However, from a practical standpoint, the differentiation of these 2 forms is of no significance at present.

Form 19. Collected 10 times; rather widely distributed but, apparently, not predominant in any particular locality.

Form 21. Rather generally distributed and fairly common. Collected twice in Mexico, 7 times in Texas, and 57 times in the other States of the United States. One collection made in Canada.

Form 23. Collected only in Mexico. It was found 3 times.

Form 29. Found only 4 times; twice in Iowa and once each in Texas and Minnesota.

Form 30. Isolated once, from a collection made in Wyoming.

Form 32. Collected 14 times. Found once in Mexico and in 8 States of the United States, though not very common in any particular locality.

Form 34. Collected once in South Dakota.

Form 36. Collected 96 times. Found once in Mexico and 5 times in Texas. The most common form found in Iowa, Kansas, Minnesota, Missouri, North Dakota, South Dakota, and Wisconsin.

Form 38. Of the 61 identifications from Mexico, 39 were form 38. This form was found 20 times in Texas out of a total of 42 isolations made from that State. It was also the most common form found in Oklahoma and Indiana and was found in 12 other States.

Form 39. Found twice in Mexico but not in Texas. Collected 20 times in the United States; apparently, most prevalent in Iowa, Kansas, and Nebraska.

Form 49. Collected 29 times. Four of these collections were from Mexico. Found 4 times, each, in Texas and Oklahoma and present in 10 other States.

Form 50. Found once in Mexico but not in the United States.

Form 52. Collected once in Iowa, once in Minnesota, once in California, and once in Mexico. First found in the United States, in Arizona, 1927.

Form 56. Found twice in Iowa; for the first time in the United States; isolated in 1927 by the Dominion Rust Research Laboratory.

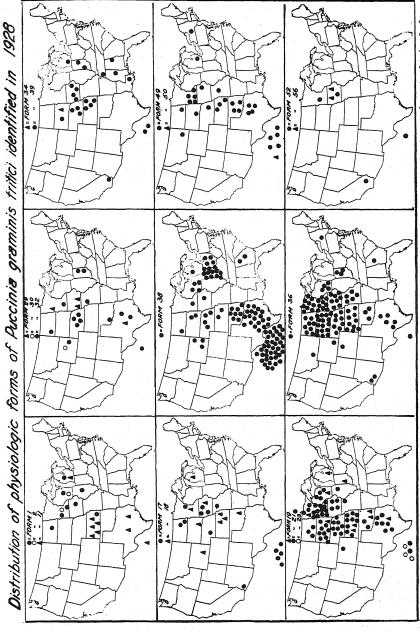
Identity and nature of isolated physiologic forms. The reactions of 12 differential varieties to the forms isolated in 1928 are as follows: Little Club was susceptible to all forms isolated in 1928. The vulgare wheats, Marquis, Kanred, and Kota, were susceptible to 13, 8, and 16 of the forms,

TABLE 8.—Prevalence and distribution of physiologic forms of Puccinia graminis tritici isolated in North America in 1928

United States Alabama Arizona California Colorado Georgia Illinois Indiana Iowa I Kansas I Louisiana Michigan Minesota Montana Nebraska New York North Dakota Ohio Oklahoma South Dakota Tennessee Texas Wisconsin Wyoming Canada Manitoba Mexico Coahuila	1 1 1 3 5 3	i	 1 2	Num 1 1 4 9				isol	ated 2 1 1 3 1 8	1 1 1 1	49		1		Cultures 1 4 2 2 3	Forms 1 3 2 2 2
Alabama Arizona California Colorado Georgia Illinois Indiana Iowa I Kansas Louisiana Michigan Minesota Mississippi Missouri Montana Nebraska New York North Dakota I Ohio Oklahoma South Dakota Tennessee Texas Wisconsin Wyoming Canada Manitoba Mexico Coahuila			 1 2 	 1 1 4 9 			 2		 2 1 1 3 1	1 1 2 2 2					1 4 2 2	1 3 2 2
Alabama Arizona California Colorado Georgia Illinois Indiana Iowa I Kansas I Louisiana Michigan Minesota Mississippi Missouri Montana Nebraska New York North Dakota I Ohio Oklahoma South Dakota Tennessee Texas Wisconsin Wyoming Canada Manitoba Mexico Coahuila			 1 2 1	1 1 4 9 		2	 2 		2 1 1 3 1 8	1 1 2 2 2					$\frac{4}{2}$	3 2 2
Arizona California Colorado Georgia Illinois Indiana Iowa I Kansas Louisiana Michigan Minesota Mississippi Missouri Montana Nebraska New York North Dakota I Ohio Oklahoma South Dakota Tennessee Texas Wisconsin Wyoming Canada Manitoba Mexico Coahuila			 1 2 1	1 1 4 9 		2	 2 		2 1 1 3 1 8	1 1 2 2 2					$\frac{4}{2}$	3 2 2
California Colorado Georgia Illinois Indiana Iowa I Kansas I Louisiana Michigan I Minnesota Montana Nebraska New York North Dakota I Ohio Oklahoma South Dakota Tennessee Texas Wisconsin Wyoming Canada Manitoba Mexico Coahuila			 1 2 1	1 1 4 9 		2	 2 		 1 1 3 1 8	1 2 2 2					2 2	2 2
Colorado Georgia Illinois Indiana Iowa I Kansas I Louisiana Michigan Michigan I Minnesota Montana Nebraska New York North Dakota Ohio Oklahoma South Dakota Tennessee Texas Wisconsin Wyoming Canada Manitoba Mexico Coahuila	3 5 1		 1 2 1	1 1 4 9 		2	 2 		1 1 3 1 8	2 2 2					2	2
Georgia Illinois Indiana Iowa I Kansas I Louisiana Michigan Michigan Minnesota Missouri Montana Nebraska New York North Dakota Ohio Oklahoma South Dakota Tennessee Texas Wisconsin Wyoming Canada Manitoba Mexico Coahuila	3 5 1		 1 2 1	 1 4 9 		2	 2 		1 3 1 8	2 2 2						
Illinois 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	3 5 1	3 1 3 	 2 1	 4 9 		2				 2 2		 			3	0
Indiana Iowa 1 Kansas 1 Louisiana 1 Michigan 1 Minesota 2 1 Mississippi 1 Missouri 1 Montana 1 Nebraska 1 New York 1 North Dakota 1 Ohio 1 Oklahoma 1 South Dakota 1 Tennessee 1 Texas 1 Wyoming 1 Canada 1 Manitoba 1 Mexico 1 Coahuila 1	3 5 1	3 1 3 	 2 1	 4 9 		2			3 1 8	2 2	- I					Z .
Iowa 1 Kansas 1 Louisiana 1 Michigan 1 Minnesota 2 1 Mississippi Missouri 1 Montana 1 Nebraska 1 Ohio Oklahoma South Dakota 1 Tennessee Texas Wisconsin 1 1 Wyoming Canada Manitoba 1 Mexico Coahuila 1 Mississippi Mississipp	3 5 1	3 1 3 	 2 1	4 9 		2			8						2	2
Kansas 1 Louisiana Michigan 1 Minnesota 2 1 Mississippi Missouri Montana Nebraska New York North Dakota Ohio Oklahoma South Dakota Tennessee Texas Wisconsin Wyoming Canada Manitoba Mexico Coahuila	5 1	3 1	2 1	9						1 4			***		20	5
Kansas 1 Louisiana Michigan 1 Minnesota 2 1 Mississippi Missouri Montana Nebraska New York North Dakota Ohio Oklahoma South Dakota Tennessee Texas Wisconsin Wyoming Canada Manitoba Mexico Coahuila	 1	. 1	2 1								1		1	2	28	11
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T) 11					_				 T	$\frac{6}{3}$ $\frac{1}{2}$		•••	1	•••	$\frac{10}{21}$	5
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Queretaro	1 8	٠	• •••				•••	***	•••			•••	,		•	
Number times each	1 8											-				
form was found 8 2	1 8		10	67	3	4 1	14	1.0	6 10	5 29	20	1	4	2	401	19

respectively. The durum varieties, Arnautka, Mindum, and Spelmar, acted as a group and were susceptible to 6 of the 19 forms. They reacted heterogeneously to 4 forms. Kubanka was susceptible to 9 forms and reacted heterogeneously to 9. Acme was susceptible to 14 forms and reacted heterogeneously to the other 5. Einkorn was susceptible to 15 forms. Of the 2 emmer varieties, Vernal was susceptible to 2 forms, and Khapli, to none.

Evidence of migration of rust from South to North: Comparison of southern and northern physiologic forms. The outstanding form in the wheat fields of Mexico and Texas in 1928 was form 38. Sixty-one identifi-



Distribution of physiologic forms of Puccinia graminis tritici as indicated by collections of rust obtained from different States in the United States and from Canada and Mexico and identified in 1928. Fig. 3.

cations were made from material obtained in Mexico. Form 38 was identified 39' times. In Texas this form was found in 20 of a total of 42 collections. Although not collected so frequently in the central and northern United States as forms 21 and 36, form 38 seemed to be quite generally distributed. It is the opinion of the writer and also of others who kept in close touch with the 1928 survey that the majority of the collections of form 38 made in the United States this year may have been due to a migration of this form from Mexico into Texas and then northward.

Early in March 3 collections of stem rust of wheat were made at different locations in Texas. These collections were made by Wallace Butler and were the first rust found by Mr. Butler that spring. These primary infections had produced only a few, very small pustules when collected. Upon identification each collection proved to be form 38. According to Mr. Butler, stem rust of wheat developed very slowly in Texas after the first infections were found. Later, when infections became more general, a larger number of collections were received from various points in Texas. The majority of these collections also were form 38.

In May, numerous collections of stem rust of wheat were made by H. H. Thornberry while studying rust conditions in Mexico. At this time most of the Mexican wheat had ripened and was cut, but many late fields were found. In several fields, and especially near irrigation ditches, the rust was in epiphytotic form, the infection being as high as 100 per cent. As stated previously, most of these Mexican collections were identified as form 38.

The reaction of Marquis seedlings to most of the Mexican collections of form 38 was somewhat at variance with their reaction to the original collection of form 38. This atypical reaction of Marquis was studied carefully, for it was clear that should such prove to be constant it would be of value in determining whether this form migrated to the United States.

Form 38 is described as producing a light type of infection on Marquis (5). The infection produced may sometimes range from 1= to $3-^c$, but the mean infection and the one most regularly encountered is 2=. Type 2 is characterized by the production of green islands with a small pustule in the center, and the island is surrounded by a rather sharp necrotic halo. The type 1= infection results when the pustule is extremely small and the green island is not evident. Occasionally, the pustules are considerably larger and may almost completely cover the green island. The increase in size of the rust pustule usually is associated with a chlorotic appearance of the island tissues. Although this complete range of infection may sometimes be found when Marquis is infected with form 38, the usual and rather constant reaction of Marquis to various collections of this form is the production of an ordinary type-2 pustule.

¹⁰ Annual report, Division of Cereal Crops and Diseases—unpublished.

In the Mexican collections of form 38 it was observed that the reaction of Marquis, in many cases, was not typical. Often the infection ranged from $1 \pm to 4$, which was somewhat like the type-x or heterogeneous infection produced by certain forms on the durum varieties. Particular attention was given to this reaction of Marquis during the season. The collections behaving this way were identified as form 38, although it was recognized that there might be a possibility of this being a new form. Subsequent inoculations with some of the Mexican collections proved that this behavior was due at least in some instances, to a mixture of 2 forms, the typical form 38 being 1 of them and form 49 the other. Form 49 was collected 5 times in Mexico independently of form 38.

Whether all of the above collections were in reality a mixture of form 38 and form 49 or even if they were some hitherto undescribed form, apparently this form invaded the United States and Canada from Mexico. Certain collections of form 38, which gave no such variation as the Mexican form 38, were found in the United States, but a sufficient number of collections were typical of the Mexican form 38 to constitute evidence of a northward migration of stem rust of wheat in 1928. Other forms isolated from Mexican collections were found in hardly sufficient number to be used as evidence for or against migration. Forms 23 and 50 were found in Mexico 3 times and once, respectively, but were not collected in the United States. It is possible that both of these forms came into the United States from Mexico if there was a migration of rust. The fact that Marquis, Kanred, and the commonly grown durum varieties are resistant to both forms may have made it difficult for these forms to become established. In the absence of susceptible varieties one would not expect these forms to increase very rapidly, even though they may have reached the fields of the northern United States.

Only 3 other forms were found in Texas as many as 4 or more times each. Forms 21, 36, and 49 were found there 7, 5, and 4 times, respectively. In the other parts of the United States these 3 forms ranked third, fourth, and fifth in prevalence.

Again, in 1928 certain forms were present in the northern wheat fields of the United States, which apparently were extremely scarce or entirely absent from southern fields. Only 1 of these was collected a sufficient number of times to be of significance in this study. Form 39 was found twice in Mexico but was not collected in Texas or Oklahoma. It was collected 20 times in the United States and seemed to be most prevalent in Kansas, Nebraska, and Iowa.

In general, the evidence accumulated in the 1928 survey suggests that some rust may have migrated from Mexico and Texas to the northern United States and Canada. The distribution of physiologic form 38 supports this

suggestion. Possibly forms 21 and 36 migrated to some extent from Mexico or Texas, as they were extremely common in the northern wheat fields. No doubt these 2 forms also spread in the North from barberries, as they were the most common forms in the barberry area in 1927.

Comparison of United States Survey with Canadian Survey. Complete results of the survey conducted at the Dominion Rust Research Laboratory at Winnipeg in 1928 are not available. The writer was furnished with an incomplete report through the kindness of Margaret Newton and Thorvaldur Johnson of that laboratory. These data show that over 400 collections made in 1928 had been identified up to January, 1929. Forms 21 and 36 were extremely common, as they comprised almost \(\frac{3}{4}\) of the identifications. Form 38 ranked third in prevalence with form 15 a close fourth. Forms 15 and 52 were fairly common, especially in the Province of Manitoba. That these last 2 forms did not spread into Canada from the wheat fields of the United States, especially those of the Mississippi Valley, is quite evident. Form 15 was not collected in the United States and form 52 was found only twice.

Whether forms 21 and 36 migrated from Texas northward is a matter of conjecture. These forms did not seem to be very prevalent in Mexico. They were, however, more prevalent in Texas, and extremely common in the northern United States. Form 38 was found rather commonly in Canada and the Canadian investigators also observed the atypical reaction of several of these collections. The same variation of the reaction of Marquis was observed by them as was noticed by the writer and others at the Minnesota laboratory.

Form 39 apparently was not present in Canada. The records show that this form has never been isolated at the Canadian laboratory. It was relatively common in the United States in 1928. If wheat stem rust migrated from the northern United States into Canada this year, the absence of form 39 in Canada may have been due to the fact that it was not present in Montana, North Dakota, Minnesota, or Wisconsin, the 4 border States of the spring-wheat region. The absence of form 39 from Canada can hardly be explained by the fact that Marquis is resistant to this form, since this would hold true for form 38 as well.

PHYSIOLOGIC FORMS EXPECTED FROM BARBERRIES IN 1928

Since form 21 and form 18 or 36 were the prevailing forms in 1927, it would be expected that these forms would develop on barberries in 1928 in greater proportion than other forms found in the fields in 1927. In the barberry area forms 21 and 36 were the most common forms in 1928. From the 1927 survey one might also expect forms 39 and 49 to originate to some extent on barberries in 1928. Form 39 was collected 7 times in Ohio in

1927 but was not collected there in 1928. Seven collections were made from Kansas in 1927 and 4 in 1928. It was not found in Iowa in 1927 but was collected there 4 times in 1928. This form was collected 33 times in 1927 and 22 times in 1928. Form 49 was collected 9 times in 1927 and, with the exception of 1 collection from Oklahoma, it was not found south of Kansas and Missouri. In 1928 this form was collected 29 times, but 13 of these collections were made in Mexico, Texas, and Oklahoma, and it is probable that, at least, these southern collections of form 49 in 1928 did not originate from aeciospores from barberry bushes. Forms 32, 17, 11, and 1 were found at various places in the barberry area in 1927, and, although they were not collected so often as forms 21, 36, 39, and 49, they were common enough in the uredinial stage to suggest that they would reappear in 1928 through the agency of the barberry. Form 38 was particularly prevalent in Ohio in 1927 and should have been one of the forms spreading from barberries in that region in 1928, if conditions were favorable for its sur-This form was collected in Ohio 4 times in 1928. Whether or not it developed there on barberries was not determined.

Forms 32, 17, 11, and 1 were found in about the same proportion in 1928 as in 1927.

In the spring of 1928 an attempt was made to identify physiologic forms that occurred naturally on barberries. Over 200 collections of aecial material were sent in by various State leaders of barberry eradication and barberry scouts. This attempt was not very successful, as it was extremely difficult to obtain fresh, viable material. When a collection was received, the infected barberry leaves were placed on moist filter-paper in Petri dishes and left until the discharge of acciospores began. Seedling wheat, oats, barley, and rye were then inoculated. No other grass hosts were available for inoculation. Sixty-two rust cultures were obtained from a total of 213 aecial collections, from which inoculations were made. Thirty-eight of these successful inoculations proved to be Puccinia graminis secalis; 2 were P. graminis avenue; and 17 cultures of P. graminis tritici were obtained. Table 9 gives the results of the identifications of the cultures of P. graminis tritici obtained from rusted barberries. Also 2 uredinial collections made near infected barberries in Indiana and 1 made in Wisconsin are included in this table. The fact that a large majority of the aecial collections gave cultures of P. graminis secalis does not necessarily mean that this strain of rust was most prevalent on barberries. It is entirely possible that the aeciospores of P. graminis secalis are more resistant to drying and to aging than those of other varieties of stem rust.

The number of identifications of physiologic forms of *Puccinia graminis* tritici from accial material was too small to be of much significance. It will be noted, however, that all the forms that occurred in the uredinial stage

TABLE 9.—Physiologic forms of Puccinia graminis tritici identified in 1928 from aecial collections and from uredinial infections directly traceable to infected barberries

Ct-1.	TT4	Numbe	er of			Phys	siolo	gic	forn	ns	
State	Host	Collections	Cultures	1	11	17	32	36	38	39	49
Iowa	Barberry	1	1	1							
Kansas	66	1	1	1							•••
Michigan		4	5	1	1				1	1	1
Minnesota		3	4	2			1	1			
Missouri		3	4			1	1	2			
New York	"		2					1			1
Indiana	Wheat near barberries	2	3				· · · · · · · · · · · · · · · · · · ·	1	2		
Wisconsin	Hordeum jubatum near bar- berries	1	1						1		
Tota	1	16	21	5	1	1	2	5	4	1	2

with any regularity in the barberry area, in 1927, with the exception of form 21, were identified from barberry collections made in 1928.

It is of interest to note that 2 of 3 collections from rusted wheat near barberry bushes in Indiana proved to be form 38 and that this form was found in 10 other collections out of a total of 20 from Indiana.

Incomplete records from the Dominion Rust Research Laboratory show that most of the forms common there in 1927 were collected in approximately the same proportion in 1928. Some forms found in Canada in 1927 had not been identified there in 1928 when these data were obtained by the writer. Other forms had been collected in 1928 that were not collected in Canada in 1927, and this would suggest some other source than local barberries.

It is, of course, unsafe to forecast the occurrence of different physiologic forms in a particular year because of the fact that they were abundant the previous year. The writer fully appreciates that there may be numerous factors influencing the survival of teliospores from one season to the next. There may be differences in the individual physiologic forms in this respect. Before it will be safe to make such a forecast a study of individual form differences and of the origin of physiologic forms must be completed. A systematic survey of the forms developing on barberries is needed each year and, until this is done, much of the evidence accumulated on the question of the source of inoculum must necessarily remain circumstantial.

DAMAGE FROM STEM RUST OF WHEAT IN 1926, 1927, AND 1928

The estimated losses, in bushels of wheat, from stem rust in 1926, 1927, and 1928, are shown in figure 4. These estimates were obtained from the Conference for the Prevention of Grain Rust.¹¹ These data include only the losses from wheat stem rust in the barberry-eradication area. In all 3 years most of the rust damage was in the hard-red-spring and durum-wheat States. In only 1 of these 3 years, namely, 1927, did stem rust of wheat

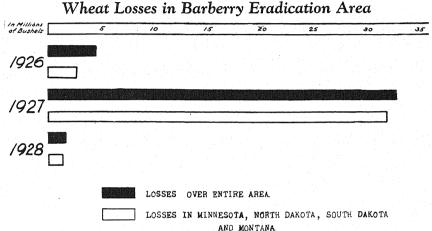


Fig. 4. Losses in terms of bushels of wheat caused by stem rust in the years 1926 to 1928, inclusive.

occur in epiphytotic form. The loss in 1926 was almost entirely due to local epiphytotics. In 1927 there was a rather general epiphytotic of stem rust and a consequent loss of over 30 million bushels. In 1928 the entire loss from stem rust was almost negligible.

DISCUSSION AND CONCLUSIONS

The evidence accumulated from the distribution and prevalence of physiologic forms of wheat stem rust in 1926 suggests that in this particular year there was no appreciable migration of inoculum from the South to the North. This would mean that most of the rust in northern fields in 1926 came from barberries in that region.

In 1927 the physiologic forms that were predominant in Texas were likewise predominant in the North, being generally distributed and extremely common. Two forms, namely, 18 and 21, appeared to be largely responsible for the epiphytotic of 1927. These 2 forms were very closely asso-

¹¹ The writer wishes to express his thanks to Mr. Donald G. Fletcher, Executive Secretary of the Conference for the Prevention of Grain Rust, for these data.

ciated in their distribution and in a great many instances were found together, either on the same rusted wheat or grass plant or in the same or adjoining fields. Collectively, they were able to attack all of the commonly grown wheat varieties.

In 1928 form 38 stood out as the most prevalent southern form. It was present in epiphytotic proportions in certain wheat fields in Mexico and was identified 39 times out of 61 Mexican isolations. As has been described previously in this paper, some of the Mexican collections of form 38 were not typical of the form 38 previously encountered in wheat-stem-rust surveys. The Mexican collections often produced an infection on Marquis wheat resembling a type-x or heterogeneous infection, later proving in several instances to have been due to a mixture of forms. Marquis is resistant to the normal form 38, developing a type-2 infection. Later in the season this atypical form 38 was found in the Northern States, but it was not so common as certain other forms. Collections of typical form 38 also were found in the Northern States, and in 3 instances it was identified from infected plants growing near infected barberries. In 1927 form 38 was rather common in Ohio and other eastern States and one would expect to find it the next year on barberries.

Form 38 is not considered a virulent form of stem rust in the hard-red-spring and durum-wheat regions, since Marquis is resistant and the durum varieties react heterogeneously to it. It could not produce an epiphytotic in the hard-red-spring and durum regions. Although the Mexican collections of this form occasionally produced what appeared to be a virulent type of infection on seedling leaves of Marquis wheat, the prevailing infection was of the resistant type. At the time this paper was prepared no study had been made of the reaction of more mature plants in Marquis to this Mexican form 38.

It is now generally accepted that the wind is an important agent in the dissemination of rust spores. A discussion of the wind and its rôle in the spread of rust spores is not included in this paper, since it has been discussed in considerable detail recently by Lambert (4).

It is the writer's belief that in 1928, at least, a limited amount of inoculum was blown into the wheat fields of the northern United States from southern fields and that the inoculum originally came from Mexico. Whether the inoculum came with the winds directly from Mexico or Texas or reached the northern fields after a gradual migration the writer is not able to say. It is believed, however, that form 38 would have been collected more times than it was had there been more susceptible varieties grown in the spring-wheat area and had an effort been made to identify the forms causing weak infections on Marquis wheat in the spring-wheat region. Naturally, the majority of collections were on Marquis or some of the durum varieties, and the average field man in making rust collections selects heavily

rusted material. These factors, no doubt, lessen the chances of the collection of forms to which the common varieties of wheat are resistant.

The statistics on the loss from stem rust of wheat show that in 1927 a rather severe epiphytotic occurred. This supports the contention of the writer that with the eradication of barberries severe epiphytotics are more apt to result when conditions are favorable for the migration of inoculum of virulent forms from the South to the North, for in this year only, of the 3 years under discussion, was there circumstantial evidence of a migration of virulent physiologic forms of stem rust. Future research on the epiphytology of stem rust may strengthen or disprove this theory but, for the present, the evidence so far accumulated corroborates the contention that in certain years the southern source of inoculum may be the most important contributing factor in the production of stem-rust epiphytotics in the northern United States and Canada.

SUMMARY

- 1. Extensive annual surveys of the prevalence and distribution of physiologic forms of *Puccinia graminis tritici* were made in 1926, 1927, and 1928 with the primary object of studying the migration of stem rust of wheat from South to North.
- 2. Evidence accumulated in 1926 suggests that there was no appreciable South-to-North migration of wheat stem rust in 1926, since the predominant forms in Texas were not found in the northern Mississippi Valley.
- 3. The loss from stem rust in 1926 was slight and only localized epiphytotics occurred.
- 4. In 1927, forms 18 and 21 were exceedingly common in Texas and were generally distributed over the fields of the northern Mississippi Valley. The evidence suggests a rather general northward migration of stem rust of wheat in 1927.
- 5. A serious epiphytotic of stem rust of wheat occurred in the springwheat region in 1927. The loss was over 30 million bushels.
- 6. In 1928, form 38 was outstandingly predominant in Mexico. In Texas it also was by far the most common form, and throughout the United States was widely distributed. The evidence was rather striking that this particular form could have originated in Mexico in 1928. It is suggested that the failure of this form to become sufficiently established to produce an epiphytotic was due to the fact that its host range is somewhat limited, as Marquis is resistant and the commonly grown durum varieties react heterogeneously to it.
- 7. The loss from stem rust of wheat in 1928 was insignificant. The total loss in the 13 States of the barberry-eradication area amounted to only $1\frac{1}{3}$ million bushels compared with nearly 35,000,000 bushels in 1927.
- 8. The value of physiologic-form surveys in the study of the epiphytology of stem rust of wheat is brought out and it is further stressed that,

along with surveys of this kind, which include principally field collections of uredinial material, similar surveys that include aecial infections are essential. Surveys of the past several years suggest rather definitely that certain physiologic forms exist in the northern United States and Canada probably exclusively through the agency of the barberry.

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THE RELATION OF PENTATHIONIC ACID AND ITS COM-PONENT CONSTITUENTS TO THE TOXICITY OF SULPHUR FUNGICIDES¹

O. NEAL LIMING²

The toxicity of sulphur to fungi has been attributed to a number of factors. Only four of these, volatilized sulphur, sulphur dioxide, hydrogen sulphide, and pentathionic acid, are now usually considered.

This report includes the results of experiments on pentathionic acid with reference to its chemical and physical properties and its relations to the effectiveness of sulphur fungicides. Vaporized and particulate sulphur, sulphur dioxide, and hydrogen sulphide are considered component constituents in the formation of pentathionic acid. The occurrence of these constituents in sulfur fungicides, before their application to host plants and to fungi and when in contact with them, was determined, and their toxicity to various fungi was tested. The conditions under which pentathionate ions are toxic to spores of *Sclerotinia cinerea* (Bon.) Schröt. also were studied.

VAPORIZED SULPHUR

In recent years renewed interest has been taken in the study of the volatilization of sulphur and its fungicidal significance. In 1920, Barker and associates (2) supported the generally accepted theory that the fungicidal action of sulphur is due to sulphur vapor. Barker (1) continued the work and reported in 1927 that the toxic agent is not gaseous but is particulate in nature. Goodwin and Martin (4) found that sulphur is given off as a gas from the parent mass; but is not toxic to fungi in that state (5).

Nearly every investigator has been interested in the claim that sulphur exerts a toxic influence at a distance. Sulphur vapor probably plays an important part in this phenomenon, in that any condensation product would likely be evenly distributed and finely particulate in nature. The result of such processes would be a coating of very fine sulphur over the plant to which sulphur has been applied. Therefore, the conditions under which sulphur vapor is produced and the toxicity of it and related products seem important to this subject.

- ¹ Abridgment of thesis submitted as partial requirement for degree of Doctor of Philosophy in Plant Pathology in the Graduate School of the Ohio State University.
- ² Grateful acknolwedgments are made to Dr. H. C. Young, at whose suggestion these studies were undertaken at the Department of Botany and Plant Pathology, Ohio Agricultural Experiment Station, and to Dr. W. G. Stover for his helpful suggestions and criticisms throughout the preparation of the manuscript.

The physical and chemical nature of volatilized sulphur. The apparatus illustrated in fig. 1 was used in the following experiment. Five grams of

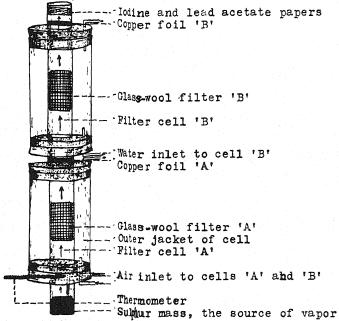


Fig. 1. Apparatus for testing the physical and chemical nature of volatilized sulphur.

sulphur were placed in the lower part of the central cell. Air washed in sulphuric acid was passed in through the air tube. A wad of glass wool was placed in the central chamber of each of the filter cells. The temperature of each filter cell was regulated by the circulation of either hot or cold water through the outer jacket. A bright copper foil was placed above the filter in each cell so that if any sulphur product passed the filter it could be detected by the formation of a tarnish on the foil. Iodine and lead acetate papers were placed at the upper end of the cells in order that the presence of sulphur dioxide and hydrogen sulphide could be detected. The conditions and results of the experiment are given in table 1.

At the lower temperatures only volatilized sulphur was formed and it passed through the hot filters. It thus appears that the volatilization product, at ordinary temperatures, is pure sulphur, of gaseous rather than particulate nature. The results of these tests agree with those of Goodwin and Martin (4).

The effect of temperature on the vaporization of sulphur. The rate of formation of tarnish on copper was used as an indication of the amount of vapor given off from a sulphur mass. The results given in table 1 show

	1,100				
Temperature at which vapor was formed	'A' filter cell	Tarnish on copper foil 'A'	'B' filter cell	Tarnish on copper foil 'B'	Test for SO ₂ and H ₂ S
$\circ c$.					
90	Hot	Trace	Hot	None	Negative
90		"	Cold	"	"
115		Medium	Hot	Medium	"
115		"	Cold	None	"
180		Heavy	Hot	Heavy	"
180	"	"	Cold	Trace	Positive
440	Cold	""	Cold	Heavy	"

TABLE 1.—The physical and chemical nature of volatilized sulphur at temperatures ranging from 90 to 440° C.

that at temperatures of 115° and 90° C. any tarnish formed is due entirely to sulphur vapor. Preliminary tests showed that a copper foil when placed about 4.5 cm. from a mass of sulphur at 93° C. would form a gray mottle in 1 minute. For convenience in tabulating the results, all of the following tests were made at 4.5 cm.

A simple cell (Fig. 2) was made by sealing a small glass shell in the

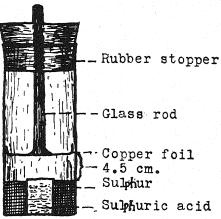


Fig. 2. Cell used to determine the rate of vaporization of sulphur and the toxicity of sulphur vapor to fungus spores.

bottom of a large tube. Copper foil was mounted on a glass rod that passed through a rubber stopper the size of the large tube. The sulphur was placed in the bottom of the cell. The small shell was filled with sulphuric acid to eliminate the effect of moisture on the tarnishing of the copper. At the start of the tests the cells, mounted copper foils, sulphuric acid, and ground sulphur were brought to the temperature desired and then quickly assembled. Each test was made in triplicate.

TABLE 2 .- The effect of temperature on the rate of vaporization of sulphur

Temperature	Number o	f hours for tarnis	h to form	Relative
remperature	Cell No. 1	Cell No. 2	Cell No. 3	rates
° C.				
24	310.0	326.0	342.0	1.0
26	179.0	171.0	163.0	1.9
28	98.0	95.0	95.0	3.4
30	64.0	62.5	64.0	5.1
32	47.5	47.0	47.0	6.9
35	32.0	32.0	32.0	10.2
40	15.9	17.6	16.9	19.4
50	3.9	4.2	79.0	
	Number of	minutes for tarn	sh to form	
60	61.0	60.0	62.0	317.0
70	15.0	15.0	15.5	1,304.0
80	3.8	4.0	3.8	4,910.0
93	1.0	1.0	1.0	19,560.0
	<u> </u>			41

The results of the tests are given in table 2. The relative rates of vaporization of sulphur at different temperatures are given in the column to the right. In the range of temperatures (24 to 35° C.) that commonly occurs during the summer the rate of vaporization varies from 1 to 10. At the higher temperatures the rate increases enormously, an increase of 70° C. (from 23 to 93°.), increasing the rate approximately 20,000 times.

The condensation and crystallization of sulphur vapor. It was observed that when sulphur was vaporized at 93° C. the vapor condensed in a very fine solid state and rapidly changed to rhombic crystals; but, when volatilized at a higher temperature (200° C.), the droplets were large and did not crystallize within 24 hours.

Sulphur, vaporized at 93° C., was condensed on a slide until the slide was milky white. Microscopic observations showed that the slide was covered with minute noncrystalline bodies. The first photomicrograph in figure 3 was taken immediately after the condensation of the vapor. In it is shown the even coverage that is often alluded to in the application of sulphur to plants by the vaporization method. The next picture of the same field was taken 30 minutes later, the third one an hour later, and the fourth, after 5 hours. Some of the drops, because of their smaller surface curvature and lower vapor pressure, apparently absorbed the vapor from the smaller drops. The larger drops then solidified and crystallized into rhombic sulphur.

The effect of light, moisture, and physical condition on the vaporization of sulphur. In these experiments the loss of sulphur from a sulphured

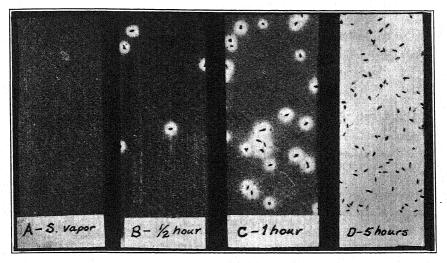


Fig. 3. The crystallization of condensed sulphur vapor on a glass slide.

glass surface by vaporization was determined by direct weighing. Ten watch crystals were arranged one above the other and held apart by small glass rings. Thus a glass tower with a large surface area (750 sq. cm.) was formed. These towers were hung in an atmosphere charged with the sulphur to be tested until each was covered with about 0.2 gm. of sulphur. They were then exposed to the various conditions mentioned above and weighed at different intervals to determine the amount of sulphur lost by vaporization.

The general results of the tests and the conclusions are all that are considered necessary to give here. The amount of sulphur lost in a dry atmosphere was not materially different from the amount lost in a humid atmosphere. The tests on the effect of light on the vaporization of sulphur were not entirely satisfactory because of the inability to control the temperature of the sulphur exposed to sunlight. It is believed, however, that light has but little, if any, effect on the rate of vaporization of sulphur. Tucker (8) and Goodwin and Martin (4) likewise concluded that these conditions have no significant effect on the rate of vaporization of sulphur.

To test the effect of the physical condition of sulphur on vaporization, flowers of sulphur, ground sulphur, and vaporized sulphur were used. The initial rate of vaporization of the vaporized sulphur at 50° C. was much greater than that of the other two types. It appears that the more finely divided crystalline form (condensed sulphur vapor) vaporizes more readily than ground sulphur and flowers of sulphur, both of which contain small amounts of amorphous sulphur.

The toxicity of sulphur vapor to fungus spores. As stated earlier, sulphur may exert a toxic influence at a distance. The assumptions are that the vapor acts directly on the fungus or that the condensation products of the vapor are toxic.

To test the toxicity of the vapor given off from a sulphur mass, cells like the one illustrated in figure 2 were used. The copper foil was removed and the round end of the glass rod was left in its place. Preliminary tests showed that spores of Sclerotinia cinerea and Cladosporium fulvum (Cke.) were not killed at a high temperature (55° C.) so readily in a dry atmosphere as when moisture was present. For this reason sulphuric acid was placed in the small shell. It was necessary to vary the temperature in the experiment to change the amount of vapor formed. The cells were first exposed in the oven until they reached a constant temperature. The end of the glass rod was touched to the dry fruiting surface of a fungus colony, and the cell was then assembled as in the previous experiment. They were then exposed to various temperatures for different lengths of time. Checks without sulphur were run at the same temperatures and under the same condition in each of the tests. After exposure to the sulphur vapor, the spores were transferred to a drop of water for germination. At the end of 16 hours the percentage of germination was determined by direct counting of the spores.

The conditions of the experiment and the results are recorded in table 3. The percentage of germination was quite variable; apparently, some spores being more sensitive to a high temperature than others in the same culture. The results are, however, sufficiently constant to indicate that sulphur vapor

TABLE	3.— The	toxicity	of	sulphur	vapor	to	fungus	spores

Temperature at which vapor	Minutes	Percentage o	f germination
was formed	of exposure	Treated spores	Untreated spores
$\circ C.$	Sclerotin	ia cinerea	
30	20	90.3	92.3
40	10	69.0	78.1
55	5	50.5	46.7
65	2	65.7	62.9
	Cladospor	ium fulvum	
45	2	42.0	46.0

was not toxic to the fungus spores. Although the amount of sulphur vapor was increased at 65° C. to about 100 times the amount formed at 30° C. no toxicity due to the sulphur vapor was apparent. Goodwin and Martin (5) found sulphur vapor was not toxic to mildew fungi.

The toxicity of the condensation products of sulphur vapor. Even though sulphur vapor is not toxic to fungus spores, it is still possible that the condensed vapor or recrystallized sulphur is toxic or that it is chemically reactive and readily forms the toxic factor. To test this point sulphur vapor was condensed on glass slides until they were milky white. Drops of water containing spores were placed on the slides and they were then inclosed in a Petri dish and left for a 16-hour germination period. In other tests the slides were left exposed to laboratory conditions for 24 hours before the water and spores were added.

The results are given in table 4. The condensed sulphur vapor or recrystallized sulphur did not prevent germination of spores of *Sclerotinia cinerea*. However, through the formation of the toxic factor, the recrystallized sul-

TABLE 4.—The	toxicity of co	ndensed	sulphur	vapor,	at	various	lengths	of	time	after
	vaporizati									

Temperature at which vapor was formed	Hours after vaporization	Percentage of germination
$\circ C$		
80	0	89.4
80	24	27.4
110	0	91.0
110	24	12.2
200	0	75.7
200	24	0.9
Check on untreated slide		88.8

phur became toxic on standing and reduced the percentage of germination below that of the check in all tests.

SULPHUR DIOXIDE

The theory that sulphur dioxide is the toxic factor of sulphur fungicides has not been supported by recent investigations. Williams and Young (10) found only minute traces of sulphur dioxide associated with ground sulphur. Liming and Young (6) showed that a much greater concentration than is found in ground sulphur is necessary for any significant toxicity to spores of Sclerotinia cinerea.

The oxidation of sulphur by the oxygen of the air is probably sufficient to account for the small amount of sulphur dioxide present. It is recognized that there may be formed on the sulphur particle a layer of S=0 that gradually changes to sulphur dioxide by further oxidation. Sulphur dioxide appears to be a transitory product and, as such, the source of the acids of sulphur. Field experiments with sulphur dusts treated with manganese dioxide, an oxidizing agent, indicate that sulphur dioxide and thiosulphates are formed and then reduced to the toxic factor.

The production of sulphur dioxide in sulphur. Tests were made to determine what natural factors affect the accumulation of sulphur dioxide in sulphur. For each test, 25 gm. of sulphur were placed in a 50 cc. flask, treated in various ways, and then set away for 5 months under different conditions. At the end of the tests, 25 cc. of water were added to each of the flasks and the contents mixed thoroughly. Each suspension was divided into two equal parts, one was titrated directly with iodine, and the other was treated with formaldehyde and then titrated. The difference in the amount of iodine used in the two fractions indicated the amount of sulphur dioxide in the suspension.

TABLE 5.—The effect of time, light, moisture, high temperature, and oxygen on the accumulation of sulphur dioxide in sulphur

Light wet 23° C.	Light wet oxygen 23° C.	Light dry 23° C.	Light dry oxygen 23° C.	Oxygen dark wet 45° C.	Oxygen dark wet 23° C.	Oxygen dark dry 45° C.	Oxygen dark dry 23° C.	Dark wet 45° C.	Dark dry 45° C.
Xª	X	x	x	x	X	x	x	X	x
x	xxx	xx	xxx	XX	x	x	XX	z	x

ax indicates trace, xx light, and xxx a medium amount of SO2.

The symbols used in the table indicate the relative amount of sulphur dioxide present in the solutions. At the beginning of the experiment a trace of sulphur dioxide was found in checks to all of the tests. Sulphur treated with oxygen did not give consistent results unless it was exposed to light. Oxygen and light were the only factors that gave consistent evidence of increasing the amount of sulphur dioxide in sulphur.

The toxicity of sulphur dioxide to fungus spores. Tests were made to determine the sensitivity of spores of Sclerotinia cinerea to sulphur dioxide. A quantity of the gas, which had been washed in sulphuric acid, was measured into an Erlenmeyer flask. The dry spores were held on a dry glass rod, as in the toxicity tests of sulphur vapor. The flask was then closed with the stopper that held the glass rod. The spores were located as near the center of the flask as possible. The flask was continuously turned and rotated during the time of exposure. The spores were then removed and placed in distilled water for germination. At the end of the tests the gas was slowly forced into a large flask partly filled with water which took up the sulphur dioxide. The transfer was made so as to determine only the amount of gas in the atmosphere in the original flask. The amount adsorbed on the walls of the flask, and so not in the atmosphere, was not measured. The solution was next titrated with iodine.

TABLE 6 .- The toxicity of sulphur dioxide to spores of Sclerotinia cinerea

	Duration of exposure and percentage of germination									
Percentage of sulphur dioxide in atmosphere	Seconds			Minutes					Hrs.	
	10	20	30	1	2	3	5	10	5	
9.7	49.8	0.0								
4.7	87.1	60.1	12.2	0.0						
2.3	95.4	90.1	74.0	51.2	0.0					
0.97		85.4	73.7	68.0	28.1	0.0				
0.50				89.7	87.6	76.4	10.2	0.0		
0.19						90.1	87.1	57.2		
0.01									39.2	
Check in washed air			7.5						90.8	
0.01						50.1	01.			

The results are given in table 6 as the percentage of germination of spores exposed to the gas for the time and at the concentration indicated. Germination of 39.2 per cent of the spores occurred after 5 hours' exposure to a 1–10,000 concentration. The results of the tests, which lasted for only a few seconds, are probably not accurate, since it is possible that the atmosphere was not thoroughly mixed during the test. However, the results indicate that spores of *Sclerotinia cinerea*, which are very sensitive to sulphur fungicides, are probably not at all sensitive to sulphur dioxide at concentrations in which it occurs in ground sulphur.

The toxicity of sulphurous acid to spores of Sclerotinia cinerea. Moist conditions necessary for spore germination are favorable to the formation of sulphurous acid from sulphur dioxide. Therefore, toxicity tests were made with 1.5 M solutions of sulphurous acid (saturated aqueous solutions of sulphur dioxide) and with solutions as dilute as 0.07 M. The spores were germinated in smear cultures on cover glasses inverted over van Tieghem cells, as described by Liming and Young (6), except that 10 cc. of the solution tested were placed in the bottom of the germination chamber. The solutions were buffered with a H_3PO_4 -NaOH solution to eliminate the toxic effect of the hydrogen ions.

TABLE 7.—The toxicity of sulphurous acid to spores of Sclerotinia cinerea

Molecular concentration	Percentage of saturation	Percentage of germination			
1.5	100	10.5			
0.3	20	89.9			
0.2	13	92.6			
0.07	5	92.1			
Check in buffer solution		93.1			

A 0.3 M. solution of sulphurous acid, buffered to pH 5.4, was found to be the maximum concentration in which germination of spores was not inhibited. In the tests in which the spores did not germinate the toxicity can probably be attributed to the high molecular concentration of the solution. It is concluded that the sulphite ion is not toxic to spores of Sclerotinia cinerea.

HYDROGEN SULPHIDE

Barker (1), while working on the volatility of sulphur, noticed that hydrogen sulphide is formed from sulphur when dusted on living leaves. Marsh (7) confirmed this work and further observed that the gas is produced when sulphur is dusted on fungus colonies or mixed with fungus spores. Wilcoxon and McCallen (9) support the English workers.

Hydrogen sulphide does not exist in ordinary ground sulphur (10). If hydrogen sulphide is formed from the hydrolysis of sulphur in the presence of moisture it apparently reacts at once with the other products of hydrolysis and the oxides to form more complex compounds. The sulphur dioxide associated with sulphur must be reduced if pentathionic acid is formed from it. Hydrogen sulphide is the most likely reducing agent, and, although not existing in ground sulphur, it may be an important transitory product.

The production of hydrogen sulphide in ground sulphur. Several tests were made to determine the effect of natural conditions on the production of hydrogen sulphide in ground sulphur. Flasks were prepared as in the first experiment with sulphur dioxide and then treated as indicated in table 8. The presence or absence of hydrogen sulphide at the end of 5 months was determined by placing a lead-acetate paper on the sulphur mass.

TABLE	8.— The	effect	of time,	light,	moisture,	and hi	igh temp	erature	on th	ie e	accumula-
			tion of	hydro	gen sulph	ide in	sulphur				

Light wet 23° C.	Light dry 23° C.	Dark wet 23° C.	Dark wet 45° C.	Dark wet 110° C.	Dark dry 45° C.	Dark dry 110° C.	Dark dry 23° C.
0	0	0	0	0	0	0	0
0	0	0	0	x	0	-	0

0 indicates no hydrogen sulphide; x indicates a trace of the gas.

In all of the tests except one the lead-acetate tests were negative. Wet sulphur, kept in the dark 5 months, at 110° C., gave a positive test for hydrogen sulphide, but only a trace was formed. Conditions, such as light, moisture, and heat, to which ground sulphur is exposed in the field, apparently, have no marked effect on the formation and accumulation of the gas in ground sulphur.

The effect of higher plants on the production of hydrogen sulphide. When sulphur is dusted on living plants hydrogen sulphide is formed and may be given off as a gas. Tests were made to determine to what extent the gas is formed when sulphur is dusted on living leaves. A simple chamber was made by placing a rubber ring between the 2 parts of a Petri dish, thus holding them apart the width of the ring. To the ring was glued a piece of cheese-cloth, so that, when assembled, the chamber was divided into 2 parts by the cloth. A section was cut out of the rubber ring to make a hole for the petiole of the leaf. In the bottom of the chamber was placed a lead-acetate paper and to the top were glued 2 glass rings with cover-glasses. These glasses were used to hold drops of distilled water very near the sulphured leaf. The leaf was dusted with ground sulphur and then enclosed in the chamber as indicated in figure 4. The dusted leaf was not in contact

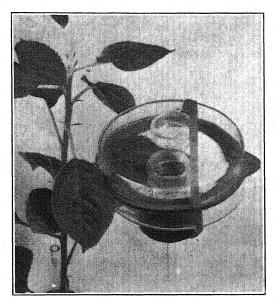


Fig. 4. Cell used in testing the toxicity of hydrogen sulphide, produced from sulphur on apple leaves, to spores of *Sclerotinia cinerea*.

with the glass or the lead-acetate paper and was only lightly supported by the cloth diaphragm. The test chambers were opened at the end of 24 hours. The drops of water on the cover-glasses were tested for a change in acidity; the change was never greater than that in drops held over non-dusted leaves. The degree of coloration of the lead-acetate paper was taken as an indication of the relative amount of hydrogen sulphide formed. The results given in table 9 show that during a 24-hour period never more than

a trace of hydrogen sulphide was produced from sulphur by any of the leaves, excepting those of the bean and strawberry.

TABLE 9.—The effect of higher plants on sulphur with reference to the production of hydrogen sulphide

	$ m H_2S$ from lphured leaves	Species of plant and side of leaf tested	$ m H_2S$ from sulphured leaves
Both sides of leaf		Lower side of leaf	
Cottonwood	x	Bean	X
Geranium	0	Strawberry	x
Apple	x	Red Oak	0
Tomato	0	Maple	0
Elm	0	Apple	x
Peach	x		
Cucumber	x	Upper side of leaf	
Strawberry	XX	Rose	x
Onion	0	Strawberry	x
Bean	XX	Bean	
Blue grass	x	Cyclamen	. x
Dahlia	x	Cucumber	0
Potato	0		

⁰ indicates none, x trace, and xx light.

The effect of fungi on the production of hydrogen sulphide from sulphur. Twenty-three species of fungi were tested to show the relation between sulphur and the type of fungus in the production of hydrogen sulphide. Phycomycetes, ascomycetes, basidiomycetes, and deuteromycetes, and saprophytic as well as parasitic fungi were included in the group. Fungi causing plant diseases controllable with sulphur and those not subject to such control were tested. These types of fungi were tested in order to gain some idea concerning the relation between the production of hydrogen sulphide by a fungus and the control with sulphur of the disease it causes.

In each test the fungus was transferred to an agar plate and allowed to grow until the colony almost filled the plate. The fungus colonies were then heavily dusted with acid-free sulphur. A lead-acetate paper was glued in the top of each of the Petri dishes. The tests were run for 3 days, at the end of which time the color of the lead-acetate papers was taken as an indication of the relative amount of hydrogen sulphide formed.

The results of the experiment are recorded in table 10. All of the fungi produced hydrogen sulphide from sulphur. However, there seems to be no correlation between the rate of hydrogen sulphide production and the type of fungus tested.

TABLE 10.—The effect of fungi on sulphur with reference to the production of hydrogen sulphide

${ m H_2S}$ from Sulphured colonies	Fungi tested	H ₂ S from sulphured colonies
Sclerotinia cinercaxxx	Alternaria sp.	XXX
S. sclerotiorum (Lib.) Bounck xxx	Fusarium lini Bolley	
Cercospora beticola Sacc xa	F. conglutinans Wollenw.	
Willow canker fungus xxx	Phoma pomi Pass.	XXX
Coccomyces hiemalis Higgins xx	Penicillium sp.	
Rhizoctonia solani Kühn xxxx	Aspergillus sp.	xxx
Cladosporium fulvumx	Phomopsis sp.	. x
Armillaria mellea Vahlxx	Glomerella cingulata (Stonem.) S	ene. Postantina de
Venturia inaequalis (Cke.) Wint. xxx	& v. S.	xxxx
Botrytis cinerea Pers xxxx	Colletotrichum lindemuthianum (S	•
Rhizopus nigricans Ehrenb xxxx	& M.) B. & C.	xx

a x indicates trace, xx light, xxx medium, and xxxx heavy.

In another series of tests, 12 fungi were treated with sulphur dusts carrying different amounts of pentathionic acid. In these tests young colonies of the fungus were dusted with the sulphur and the relative amount of hydrogen sulphide produced was determined as in the above tests. At the end of 3 days the increase in diameter of the colony and the intensity of coloration of the lead-acetate paper were observed in each case. The tests of Sclerotinia cinerea (Fig. 5) illustrate the effects of the different sulphur

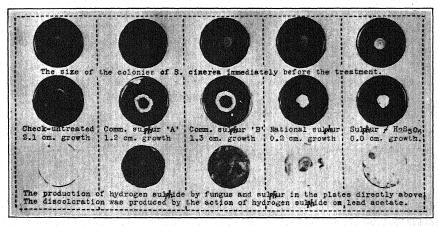


Fig. 5. The relation of hydrogen sulphide and of pentathionic acid to the growth of *Sclerotinia cinerea*, determined by treating matched colonies of the fungus with sulphur dusts loaded with various amounts of pentathionic acid and 3 days later measuring the growth of the colonies and the amount of hydrogen sulphide produced.

dusts on the growth of the fungus and the amount of hydrogen sulphide produced.

The colonies dusted with sulphur carrying a large amount of pentathionic acid were killed or stunted and produced but a slight amount of hydrogen sulphide, while those treated with sulphur with only a small amount of pentathionic acid continued growth and produced a large amount of the gas. Thus, it is concluded that the production of hydrogen sulphide from sulphur cannot be correlated with the toxicity of sulphur to the fungitested.

The toxicity of hydrogen sulphide in the gaseous condition. The results of preceding experiments indicate that hydrogen sulphide is not toxic to the fungi in the fruiting and vegetative stages. There is the possibility, however, that the gas has an adverse effect on fungus spores and affects their germination. The toxicity of hydrogen sulphide to spores of Sclerotinia cinerea was tested in the same manner as was sulphur dioxide.

The results of the toxicity tests are tabulated in table 11. Spores exposed to a 1-2,000 atmosphere for 5 hours showed no effects of the gas. Although

		Durat	ion of	exposu	re and	percer	itage o	f germ	ination	1
Percentage of H ₂ S in atmosphere	\$	Second	s		Min	utes			Hours	
	10	20	30	1	5	15	30	1	2	5
25.40	90.1	56.4	0.0	6.1						
10.40		96.2	89.8	58.0	0.0	7.6				
2.80				92.0	87.1	40.1	0.0		100	
0.97						89.0	76.8	25.9	0.0	
0.47							86.1	86.0	0.0	
0.10			1 1 2 2					92.3	89.8	
0.05										92.1
Check in distilled wat	er afte	er expo	sure to	room	air					91.5

TABLE 11.—The toxicity of hydrogen sulphide to spores of Sclerotinia cinerea

the influence was not great, traces of the gas and short exposures to concentrated atmospheres actually stimulated germination of the spores of the fungus.

The toxicity of hydrogen sulphide in aqueous solution. The toxicity of aqueous solutions of hydrogen sulphide was tested in the same manner as was the sulphurous acid. The concentration of each solution is given in the molecular concentration and the percentage of saturation. In the tests the solutions were buffered with a H_3PO_4 -NaOH solution to eliminate the toxic effect of the H-ions.

The results, recorded in table 12, show that relatively strong solutions (60 per cent saturation) of hydrogen sulphide buffered to pH 5.4 are not toxic to spores of *Sclerotinia cinerea*.

TABLE 12.—The toxicit	y of aqueou	s solutions of	hydrogen	$sulphide\ to$	spores of
Scleroti	nia cinerea	in the presen	ce of a bi	ıffer	

Molecular concentration	Degree of saturation	Germination		
	Per cent	Per cent		
0.140	100	0.0		
0.112	80	54.4		
0.084	60	93.7		
0.056	40	96.0		
0.028	20	98.1		
Check in distilled water		95.2		

PENTATHIONIC ACID

The theory that pentathionic acid is the toxic factor of sulphur fungicides was first advanced by Young (11). Young and Williams (12) showed that pentathionic acid is associated with ground sulphur and flowers of sulphur. Results given in the paper by Liming and Young (6) indicate that, of the acids of sulphur, only the polythionic acids are toxic to fungus spores. They also show that when manganese dioxide, an oxidizing agent, is added to sulphur a more effective fungicide is obtained.

The occurrence of pentathionic acid. Pentathionic acid is the principal acid in a sulphur sol formed by the reaction of hydrogen sulphide and sulphur dioxide in water. One gram of sulphur from this sol was found to carry about 0.14 millimol of pentathionic acid (3, pp. 615–625). The acid is also formed by the decomposition of a thiosulphate by an acid. This type was found to carry about 0.45 millimol of pentathionic acid per gram of sulphur (3). The acidity of pure ground sulphur is due almost entirely to sulphuric and pentathionic acids. One gram of National 300-mesh sulphur was found to carry about 0.0002 millimol of pentathionic acid. The amount of acid carried by the different types of sulphur may be varied greatly from the figures given above by varying the methods of preparation and the size of the sulphur particles.

The preparation of pentathionic acid. Solutions of pentathionic acid for toxicity tests have been obtained from the 3 sources mentioned above. Concentrated sulphurous acid was reduced with hydrogen sulphide until little but colloidal sulphur and pentathionic acid were left in the mixture. To free it from hydrogen sulphide and sulphur dioxide it was acrated for several hours until the odor of the gases had disappeared. Then the colloidal sulphur was removed by ultra-filtration. Qualitative tests showed

that only sulphur acids, principally pentathionic, with some tetrathionic and traces of sulphuric, were present. In general, 20 liters of hydrogen sulphide, when bubbled slowly into a liter of concentrated sulphurous acid, will make a 3 per cent or 0.25 N. solution of pentathionic acid.

From a solution of pentathionic acid made by the sodium thiosulphate-hydrochloric acid reaction, sodium and barium pentathionates were prepared. The salts were crystallized out in alcohol and were then taken up in water and recrystallized until, in the case of the barium salt, it was 98.6 per cent pure barium pentathionate. In 1 test, about 12 per cent of the sulphur from the thiosulphate was recovered as sodium pentathionate, and 20 per cent as the barium salt. For toxicity tests, pentathionic acid was prepared from this salt by precipitating out the barium as a sulphate.

National 300-mesh sulphur was thoroughly wet with just enough water to make a thick paste. Filtrates, which were about 0.006 N. with respect to hydrogen-ion concentration, were then taken from this paste. Quantitative tests showed that about 92 per cent of the acidity was due to sulphuric acid and 8 per cent to pentathionic acid. This filtrate was used in toxicity tests in which pure sulphuric acid of the same normality was used as a check.

The stability of pentathionic acid. There is a great difference of opinion concerning the stability of pentathionic acid. It is generally reported that strong acids and alkalies decompose the acid. Concentrated solutions of alkalies were added to concentrated solutions of the acid in the preparation of the pentathionate salts and little or no decomposition occurred. Strong alkalies when added to dilute solutions of the acid, however, destroy the pentathionate ion with the deposition of sulphur. Likewise, ammonium hydroxide destroys the acid and causes the precipitation of sulphur. Pentathionic acid, approaching the strength of concentrated sulphuric acid, has been prepared in this laboratory and seems quite stable, the only signs of decomposition being the formation of a slight amount of sulphur and sulphur dioxide. Nitric acid causes the formation of sulphur and sulphate from pentathionic acid, but this reaction is apparently due to the oxidizing properties of nitric acid. It is concluded from a consideration of these observations that pentathionic is a relatively stable acid. The barium, potassium, and sodium salts are very stable when kept at room temperatures and in a dry condition. Barium pentathionate, which has been kept in the laboratory in an open-mouth bottle for 6 months, has shown no signs of decomposition.

The volatility of pentathionic acid. The possibility that pentathionic acid is volatile was suggested by Young (11). Because of its odor and its ability to tarnish copper suspended over the acid, it appears that pentathionic acid is volatile to a certain degree. Sulphuric acid, although generally referred to as nonvolatile, will give off enough vapor to tarnish copper and to kill spores placed over a concentrated solution of the acid. Dilute

sulphuric and pentathionic acids do not show such volatility. Due to the decomposition of pentathionic acid on distillation, the degree of its volatility cannot be determined by fractional distillation.

Some indication of the volatility of the acid was obtained through reducing the volume of a solution by evaporation over calcium chloride and comparing the normality of the resulting solution with the calculated normality. If an acid is volatile the normality of the solution will not increase in proportion to the reduction of volume. After 36 hours, 2.3 per cent of a 0.25 N. solution of pentathionic acid could not be accounted for in tests in which the area of the evaporating surface was double the volume of the solution. But the possibility of any reaction that would influence the titratable acidity of the solution must be eliminated before this figure can be accepted as representing the volatility of pentathionic acid.

The toxicity of sulphur acids. The results of toxicity tests with various sulphur acids at different concentrations are presented here in table 13.

TABLE 13.—The toxicity of acid	ds of sulphur to spores of	* Sclerotinia cinerea in solutions
buffered to pH 5.4.	Results taken from Lin	ning and Young (6)

Concentration		Per	centage germi	nation	
in normality	$\mathrm{H_2SO_4}$	$\mathrm{H_2SO_8}$	$\mathrm{H_2S_2O_6}$	$\mathrm{H_2S_3O_6}$	$\mathrm{H_2S_5O_6}$
0.1000	84.2	91.1	49.8		0.0
0.0100	86.4	93.2	90.1	12.9	0.0
0.0010	96.1	92.0	90.2	20.8	trace
0.0005	94.7	95.5	89.6	74.2	12.4
0.0001	93.7	93.6	95.4	87.2	62.6
Check	93.7	93.1	94.9	92.7	92.6

For detailed descriptions of the methods and conditions of the experiment the former paper (6) may be consulted. The trithionic acid solutions were contaminated with pentathionic acid and sulphur, except when very dilute. The pentathionic acid solutions, which were prepared by the hydrogen sulphide-sulphur dioxide method, as stated earlier, contained some tetrathionic acid.

The results show that the sulphate, sulphide, and dithionate ions are not toxic, while the polythionate ions are toxic. The relative degree of toxicity appears to be in the order; pentathionate, tetrathionate, and trithionate. A 0.001 N. solution that contains about 0.013 per cent pentathionic acid prevented germination of nearly all of the sports of *Sclerotinia cinerea*, and a 0.0005 N. solution reduced germination to 12.4 per cent.

The toxicity of pentathionic acid to fungus spores. In connection with toxicity tests of other fungicides further tests have been made with penta-

thionic acid prepared from the barium salt. In these tests the spores were suspended in a solution that contained mannite in addition to the usual buffer salts. Altogether, 9 pathogenic fungi were tested. The apparatus and the methods for the tests were essentially those used in the earlier tests. The names of the fungi and the materials tested are given in table 14.

TABLE 14.—The toxicity of pentathionic acid to spores of pathogenic fungi, in a buffer-mannite solution

	Percentage of germination				
Pathogenic fungi tested	Pentath	Sulphuric acid			
	0.0068 N.	0.0068 N.	0.0068 N.		
Verticillium alboatrum R. & Ber	0.0	20.8	98.6		
Sclerotinia cinerea	0.0	60.0	97.1		
Phomopsis sp	0.0	67.5	87.4		
Graphium ulmi Schwartz	0.0	8.7	70.9		
Glomerella cingulata	0.0	71.3	91.3		
Botrytus cinerea	0.0	97.0	98.3		
Fusarium moniliforme Sheld	3.3	43.9	89.1		
Thielavia basicola Zopf.	0.0	29.1	69.9		
Phoma pomi	0.0	55.1	96.8		

With but two exceptions the percentage of germination was determined at the end of 18 hours. *Verticillium alboatrum* and *Phomopsis* sp. required 48 hours for satisfactory germination.

The results of the tests show that pentathionic acid is toxic to the spores of all of the fungi tested. The degree of toxicity to the various fungi varied considerably; this, of course, being expected. The presence of mannite in the solutions made the conditions more favorable for the germination of *Sclerotinia cinerea* spores, and, consequently, about twice the usual amount (6) of pentathionic acid was required to prevent germination.

The toxic action of sulphur at a distance. Tests were made to determine the effect of 'distance' on the toxicity of sulphur and of pentathionic acid to fungus spores. In each test a spore suspension was placed 4 mm. above the material to be tested, and in a closed cell to eliminate the effect of external conditions. The duration of the experiment was 16 hours.

The results, given in table 15, show that during a 16-hour period sulphur did not exert a toxic action at a distance of 4 mm. However, a 0.25 N solution of pentathionic acid was sufficiently volatile to prevent germination of spores in a suspension held 4 mm. above the acid. It is thus possible to account for the toxicity of hydrophilic sulphur sols at a distance (11). A more dilute solution (0.05 N.) did not exert a noticeable toxic action at the distance used in the tests.

TABLE 15.—The effect of 'distance' on the toxicity of sulphur and of pentathionic acid to spores of Sclerotinia cinerea

Materials tested	'Distance'	Germination
Dry ground sulphur	mm.	Per cent 87.6
Wet "	4	84.2
0.025 N. pentathionic acid	4	0.0
0.005 N. "	4	92.0
Check over distilled water	4	88.9

In the light of the results of this experiment and those on the production and toxicity of sulphur vapor and hydrogen sulphide, the toxicity of a sulphur at a distance cannot be attributed to any of these factors. It is believed that the movement of sulphur as a vapor and its subsequent oxidation are largely responsible for the toxic action of sulphur over a space. Hydrogen sulphide is probably an important factor in that higher oxides, which are formed from the translocated sulphur, may be reduced by it to the toxic factor.

The toxicity of sulphur filtrates. The toxicity of sulphur filtrates to spores of Sclerotinia cinerea was reported in the earlier paper (6). The tests were repeated, using 0.006 N. filtrates from ground sulphur and 0.008 N. filtrates from flowers of sulphur. The sulphurs from which the filtrates were taken were then treated with nitric acid and washed until the sulphur was neutral in reaction. Filtrates were then taken from these acid-free sulphurs, adjusted to pH 2.5 with sulphuric acid, and tested along with the first sulphur filtrates.

TABLE 16 .- The toxicity of sulphur filtrate to Sclerotinia cinerea spores

Materials used in the tests	pН	Filtrates normality	Condition of sulphur when taken	Germina- tion
Ground sulphur	2.5		Acid-free sulphur	Per cent 90.1
		0.006	Acid ''	11.0
Flowers of sulphur	2.5		Acid-free ''	89.8
		0.008	Acid '.'	7.6
Check in sulphuric acid		0.008		91.1

From the results recorded in table 16, it is concluded that sulphur filtrates, in which, as stated earlier, about 8 per cent of the acidity is due to pentathionic acid, are toxic; whereas filtrates from acid-free sulphur are not toxic to spores of *Sclerotinia cinerea*.

The effect of oxygen on the toxicity of sulphur. The results of previous tests (6) showing the effect of oxygen on the production of the toxic factor of sulphur are given here. For details of the methods and conditions the original descriptions may be read.

TABLE 17.—The effect of oxygen on the production of the toxic factor of sulphur.

Results taken from Liming and Young (6)

Materials tested	Conditions	Germination
		Per cent
National 300-mesh sulphur	Oxygen absent	0.0
Acid-free sulphur	e e e e	46.5
Check in distilled water		47.2
National 300-mesh sulphur	" present	0.0
Acid-free sulphur	a contract of	8.7
Check in distilled water		92.7

Spores in the presence of acid-free sulphur in an oxygen-free air germinated as well as when no sulphur was present. It is therefore shown that sulphur itself is not toxic to fungus spores. The results indicate that, through the natural oxidation of sulphur in the presence of the oxygen of the air and water, the toxic factor is formed.

CONDITIONS AFFECTING THE TOXICITY OF PENTATHIONIC ACID

The conditions under which sulphur fungicides are most effective have not been studied in great detail. The results of field tests made by Liming and Young (6) indicate that alkaline sulphur dusts are not so effective as acid sulphur dusts. In other tests they show that neutral solutions of sulphur compounds are not toxic, whereas comparable solutions, when acidified, are toxic.

The effect of H-ion concentration on the toxicity of pentathionate ions. Tests to determine the effect of H-ion concentration on the sensitivity of spores of Sclerotinia cinerea to the toxic action of pentathionic acid were made and reported in an earlier paper (6). The results have been rearranged and are presented here to show the relative effect of the acid ions in neutral and in acid solution.

The results which are given in table 18 show that pentathionate ions do not exert a toxic effect on the spores in neutral solutions, while in acid solutions (pH 5.4) the pentathionate ions are toxic.

The stability of pentathionate ions in the presence of fungus spores. In the preceding experiment the pentathionate ions were toxic at a reaction that is below the isoelectric range (about pH 6.0) of the fungus material, and they were not toxic at a reaction above that range. It appears, there-

TABLE 18.—The effect of H-ion concentration on the sensitivity of spores of Sclerotinia cinerea to the toxic action of pentathionate ions

Percentage germination				
Buffered at pH 5.4	Buffered at pH 7.0			
0.0	28.2			
trace	27.8			
0.0	34.2			
7.2	29.4			
72.1	22.7			
91.9	31.2			
	Buffered at pH 5.4 0.0 trace 0.0 7.2 72.1			

fore, that either the stability of the pentathionate ions or the condition of the fungus material is the controlling factor in the toxic action. The evidence so far points strongly to the latter condition.

Tests were made over a range from pH 3.0 to pH 8.4 with a 0.001 N. solution of pentathionic acid. In each test about 1 gram of spore material was placed in 5 cc. of the acid for 6 hours. By this time the spores cultured in solutions buffered to pH 7.0 had produced short germ tubes. The spore suspensions were then filtered and the spores washed with a buffered solution of sulphuric acid to remove the free pentathionic acid from the spore mass. The filtrates and the spore masses were tested for the presence of sulphide, sulphite, and pentathionate ions.

The results of the chemical tests for the various ions are recorded in table 19. In all solutions, in which hydrogen sulphide was found, the

TABLE 19.—The effect of fungus spores on the stability of pentathionate ions. O indicates none, x a trace, xx light, xxx medium, and xxxx heavy

Normal pH		Compo	unds prese filtrates	ent in	Compounds present in spore masses		
conc.		$\mathrm{H_2S}$	SO_2	S ₅ O ₆	$\mathrm{H_2S}$	SO ₂	S5O6
0.001	3.4	0	0	xxxx	0	0	xxxx
0.001	5.4	x	0	XXXX	x	0	xxxx
0.001	7.0	x	0	xxxx	0	0	xxx
0.001	8.4	x	0	xxxx	x	x	xx
Distilled wa	ter	0	0	0	0	0	0

amount was never more than a trace. Practically all of the pentathionic acid was accounted for in the filtrates and spore masses. The results indicate that pentathionate ions are relatively stable in both the alkaline and the acid conditions; that is, they are stable under conditions when they are not toxic as well as under conditions when they are toxic.

The effect of H-ion concentration on the permeability of the wall of the fungus spore. The difference in the effect of pentathionate ions at different reactions is apparently due to the condition of the spore. Tests were made to determine whether the permeability of the cell walls to the pentathionate ions is affected by the reaction of the medium. Fungus material scraped from the fruiting surface of a colony of Sclerotinia cinerea was soaked for 6 hours in dilute solutions of pentathionic acid buffered at various reactions. The fungus material was removed from the acid, washed thoroughly with distilled water, and then tested with ammoniacal silver nitrate.

The reactions at which the tests were made and the results are shown in table 20. Nearly the same amount of color, due to the reaction of penta-

TABLE 20.—The effect of H-ion reaction on the permeability of spore walls to pentathionate ions, determined by the precipitation of silver sulphide in the spores due to the reaction of ammoniacal silver nitrate and pentathionic acid

Material tested	$ \begin{array}{c} \text{Concentration} \\ \text{of } H_2S_5O_6 \end{array} $	pН	Color of inside of spores due to silver sulphide
Fungus material scraped from fruiting surface	0.001 N.	2.0 4.0	Black
of Sclerotinia cinerea colonies on potato-	((((6.0 8.0	Brown-black Black
dextrose agar	Check	6.0	Light brown

thionate ions with silver nitrate, was found in all the spores, regardless of the reaction of the solution in which they were soaked. It is concluded that the walls of spores of *Sclerotinia cinerea* are penetrated by pentathionate ions in both alkaline and acid reactions. The results of the tests indicate that the difference in the effects of pentathionates at pH 5.4 and at pH 7.0 is governed by the condition of the fungus tissue, probably the amphoteric substances, rather than by the condition of the pentathionate ions.

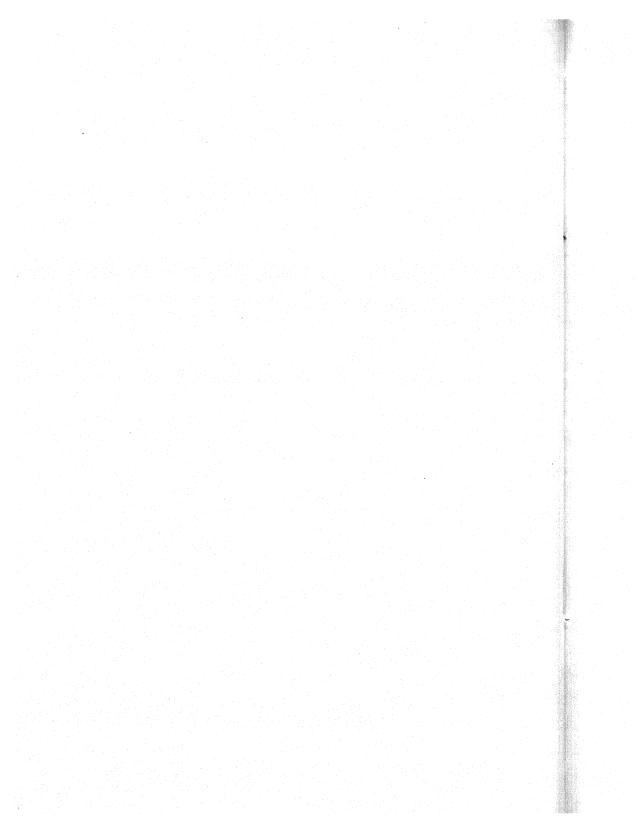
SUMMARY

- 1. The volatile product of sulphur is a vapor. The rate of vaporization is affected by temperature, a rise from 23 to 93° C. causing the rate to increase about 20,000 times. Sulphur vapor is not toxic to fungus spores, and the condensation products are toxic only after standing several hours.
- 2. Sulphur dioxide occurs only in traces in ground sulphur and is not toxic to spores of *Sclerotinia cinerea* in such concentrations. Light and high temperatures seem to favor the formation of the gas. As a transitory factor in the formation of pentathionic acid, sulphur dioxide probably plays an important rôle in the toxicity of sulphur fungicides.

- 3. Hydrogen sulphide does not occur in ground sulphur. Traces of the gas are produced from sulphur by the action of higher plants and fungi, but the gas is not toxic to fungi in such concentrations. Hydrogen sulphide is considered an active agent in the reduction of higher oxides of sulphur to pentathionic acid.
- 4. Pentathionic acid is associated with sulphur and is toxic to fungi in such concentrations. It is a natural oxidation product of sulphur, its formation being enhanced, however, by mild oxidizing agents and probably by hydrogen sulphide. It is not sufficiently volatile in dilute solutions to be toxic at a distance.
- 5. The toxic action at a distance is attributed to the combination of the following factors: the vaporization of sulphur and the oxidation of the condensed vapor that result in the formation of the toxic factor. Hydrogen sulphide is thought to be a contributing factor in these reactions.
- 6. The pentathionate ion is stable in weak acid and alkaline solutions, however, it is toxic only in acid solutions. The toxic action apparently is governed by the condition of the fungus rather than by the condition of the pentathionate ion.

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STUDIES ON THE DEVELOPMENT OF CROWN GALL, HAIRY ROOT, AND WOUND OVERGROWTHS IN TREATED SOUL^{1, 2, 3}

A. J. RIKER AND W. M. BANFIELD

INTRODUCTION

The complexity of the problem of overgrowths on apple, with the attendant difficulty of accurate diagnosis, has been an obstacle to a clear understanding of the manner of infection, development, and symptoms of the diseases in this group. A number of different workers, beginning with Smith et al. (16), have pointed out that considerable variations occurred not only in the different overgrowths but also in various organisms that may be associated with certain of them. It appeared that the understanding of this complex problem would probably be advanced if methods of experimentation were improved to the point at which each of the several types of overgrowth could be induced at will without mixture with one another. The separate development under partially controlled conditions of crown gall and callus on nursery apple trees was accomplished by Riker and Keitt (12), and by Muncie (6). The earlier studies have been enlarged and extended by the present writers, who began this work at Madison, Wis, in the spring of 1927.

A number of different experiments have been performed to follow the development not only of crown gall, caused by *Phytomonas tumefaciens* (Smith and Town.) Com. S. A. B., but also of hairy root, caused by *Ph. rhizogenes* R. B. W. K. and S., and girdle knots. These trials were conducted under conditions where efforts were made to eliminate, as far as feasible in the field, the factors that might induce development of complicating overgrowths, and to induce only the particular one under consideration. A report of these studies, mentioned in an abstract (10), is given in the present paper.

METHODS

Several methods were employed that had varying refinements in the manner of regulating possible extraneous influences. These consisted primarily in growing apple grafts in the field (1) in beds of soil that, as far as records are available, had never grown a crop naturally infected with crown

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gall and (2) in similar soil that had been steamed. Various organisms were then introduced into certain beds. Further refinements of method in which portions of apple stems were enclosed in glass cylinders will be described in a later paper.

The soil selected for steaming in 1928 was a sandy loam of good fertility that had been employed for a number of years to grow truck crops. This soil was prepared by plowing and spading to a depth of 10 in. and was steamed according to the well-known method recently described by Johnson (4), which was sufficient to destroy bacteria of the crown-gall group. mediately after steaming, 12-in, boards were placed edgewise around the beds so as to prevent washing in of surface water. Several of the beds were held as controls, while other individual beds were treated, respectively. with heavy suspensions of certain organisms under consideration. suspesions were applied to the surface and worked down through the soil to a depth of 6 in. The control beds were sufficiently separated from the inoculated beds to make remote any chance contamination. The steamed beds were situated so that water could easily be supplied when desirable during dry summer weather. Six beds were employed for these studies, in which Wealthy apple grafts were planted at the rate of 400 grafts per bed. Although rather crowded, they grew very well during the time of the experiments. As the plants developed in these beds they were sprayed at various intervals with Bordeaux mixture to repel leaf hoppers and to prevent infection with various leaf-spot organisms, particularly that of apple scab. Soil of a similar type was employed for a repetition of the experiments in 1929. The treatment of the soil was similar except that the soil was not steamed. Since there was no available supply of water for the second series, the trees in this experiment suffered somewhat from drought, especially in the summer of 1930.

The cultures employed for treating the soil were as follows: Crown-gall, No. 2018, originally isolated from a large soft gall on apple and shown to be pathogenic on a number of plants, including apple; hairy-root, No. 2014, and No. T43-1e, originally isolated from woolly-knot overgrowths on apple and found able to induce hairy root on apple; a mixture of the crown-gall and hairy-root organisms, No. 2004, originally isolated from apple and that had produced symptoms of both crown gall and hairy root on apple, tomato, and tobacco; Bacillus radiobacter, Beij. and Van Deld., No. R 11, originally secured from the soil, was nonpathogenic on any of a series of plants tried. Studies of the morphology and physiology of these cultures are reported in a paper by Riker et al. (11) and by Wright et al. (17). Since the plants treated with B. radiobacter behaved like the controls, they are listed in later pages with the controls.

The pathogenicity of the cultures employed was determined in parallel studies, as shown in table 1. In each soil bed to which organisms were

TABLE 1.—Results following control punctures and different types of inoculations below ground with crown-gall and hairy-root organisms on the scions and unions of Wealthy apple grafts grown in various soil beds

Cultures used in soil beds and for inoculations	Treatment of grafts	Trees	Crown gall	Hairy root	Negative
Experiments on scions—results		Number	Number	Number	Number
taken after growth of the					
trees during 1928 and 1929					
in steamed soila					
Hairy root, No. 2014	Inoculated	39	0	33	6
Do	Punctured	20	0	0	20
Hairy root, No. T43-le	Inoculated	30	0	16	14
Do	Punctured	20	0	10	20
D0	r unecured	20	0	U	20
Crown-gall and hairy-root mix-					
ture, No. 2004	Inoculated	36	33b	2	1
Do	Punctured	19	3	0	16
Crown gall, No. 2018	Inoculated	33	22	0	11
Do	Punctured	19	1	0	18
Control	Punctured	107	0	1	106
Experiments on unions-results					
after growth of the trees dur-					
ing 1929 and 1930 in natural					1 4 5 6
soil					
Hairy root, No. 2014	Smearede	94	0	5	89
Do	Inoculated	226	0	83	143
Do	Punctured	119	0	0	119
Control	Punctured	187	0	1	186

^a The grafts were planted in steamed-soil beds to which various organisms, respectively, were added. As indicated, a portion of them were inoculated through punctures; the others were merely punctured, as explained in the text. The final results were taken at the end of the second growing season.

applied, a number of trees were punctured, but not inoculated, and others were inoculated by means of punctures on the underground parts with the same organisms that had been earlier applied to the soil. The soil about the trees was momentarily moved aside from time to time as the season progressed, and the trees were examined to note the stages in the development of the various overgrowths. Except for addition of the bacteria, the

b Various degrees of hairy-root development were also shown by these overgrowths.

c Covered with bacteria.

control beds received the same treatment. It is noteworthy that following the punctures in the soil treated with bacteria only an occasional infection occurred. Since these bacteria live in the soil for some time (8) and (1) and were isolated from the soil in these beds subsequent to this treatment, there was no doubt of their presence. Although the bacteria were present in the soil, the question is raised whether they were able to gain entrance into the punctures before these punctures were closed as infection courts. Similar results were found in other experiments recorded and discussed later in this paper.

This series of experiments in the field is open to the criticism that, while the possibility that the crown-gall and related organisms may be present as contaminants is reduced, especially at the beginning while the wounds are open infection courts, it has not been eliminated.

In the steamed soil these organisms were doubtless destroyed by the heat, if they were present. However, since both the steamed soil and natural soil had never, as far as is known, carried a crop susceptible to crown gall, it seems unlikely that this organism was present at the beginning of the experiments. The fact that the controls in both the steamed and natural soil remained nearly free from infection is evidence in favor of this view. However, there is always the possibility that some one of a number of possible agencies might have introduced an undesired organism of this group. The organism, if thus introduced, might move even through the soil, but such movement is so slow (3) that it is probably not an important factor, especially since the organisms under consideration are wound parasites. The greatest obstacle of all in this type of work is the possibility of introducing the causal bacteria upon the roots of the apple grafts. While various antiseptics may be applied to the surface of the seedling roots at the time the grafts are made, the writers, in a long series of unpublished trials, have not yet found any means of removing, with full confidence, all such organisms from the seedling roots. This is made extremely difficult because, at the time the seedling roots are harvested, a considerable number of the small lateral roots are either injured or broken off. Very frequently they die back to their origin, thus giving rise to the formation of small The present writers are unaware of any treatment that will destroy organisms in these cavities without injury to the main root, itself. The difficulty of growing seedling roots in any case, to say nothing of growing them under sterile conditions, prompted the present writers to seek other methods of approaching this problem. The method later employed involved placing portions of the stems of naturally growing plants under aseptic conditions and carrying on the experiments with these parts. results of this work are reported in another paper.

DIFFERENTIATION OF VARIOUS TYPES OF DEVELOPMENT

The difficulty of making accurate diagnoses of the various malformations included in the complex under consideration has been considerably augmented because of the intergradations of the various symptoms and of the trouble in distinguishing one of several different stimulating agencies from the others. In the present studies, since certain particular causes of various overgrowths have been separated from one another as far as practicable, it is possible to clarify certain questions that have been raised concerning the identity of the different manifestations.

In the steamed soil beds in the field particular attention has been given to crown gall, hairy root, mixtures of crown gall and hairy root, and girdle knot. It is quite possible that the type of nonparasitic enlargements often encountered when different plants are grafted, such as those mentioned by Kostoff (5) and by Riker (9) is encountered from time to time on apple. A consideration of such overgrowths is not included in the present paper. This complication was intentionally avoided in most cases by inducing the different enlargements on the scion wood above the union.

The crown galls developed on these experimental plants, following inoculations with *Phytomonas tumefaciens*, conformed in appearance to the descriptions and illustrations that have already been given (12) and (6). The symptoms on these plants seem to correspond to those reported by Brown (2) and Siegler (14) as being induced by their "peach strain" of the crown-gall organism. The cultures used by the present writers were all isolated originally from apple.

Hairy root developed following inoculations with *Phytomonas rhizogenes* in accord with the description and illustrations by Riker *et al.* (11). Similar developments are described by Muncie and Suit (7).

Mixtures of the crown-gall and hairy-root organisms resulted in a series of overgrowths that showed crown galls with roots developing from various portions of the gall tissue. In some cases the roots came from the stem at the outer edge of the gall tissue and in others from the gall tissue proper. A rather complete intergradation between crown gall and hairy root was secured (Fig. 1, C, D). Some of the symptoms produced by *Phytomonas rhizogenes* and by a mixture of *Ph. tumefaciens* and *Ph. rhizogenes* suggested those found by Siegler (15) and Brown (2) following inoculations with their apple strain of the crown-gall organism.

Girdle knots (Fig. 1, B) were induced on the scions of the young apple trees after wrapping the stems several times around with copper wire at the time of planting. (Table 2). This was done in an effort to obtain the callus or wound-overgrowth type of manifestation that occurs following an interference with the downward flow of elaborated food from the upper portions of the plant because of failure at the graft union. At the same

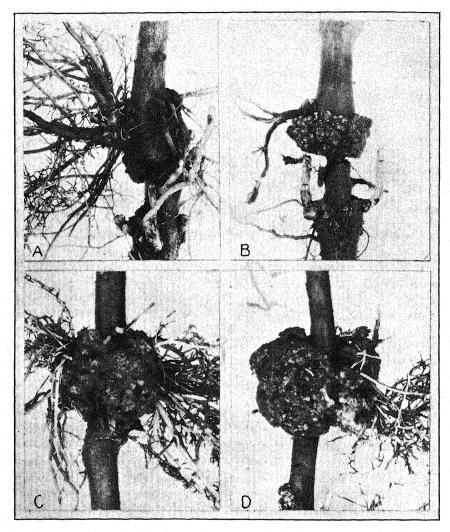


Fig. 1. Overgrowths developed in steamed-soil beds in the field after two seasons of growth. A. Noninfectious hairy root on the scion above a girdle of copper wire developed in the control bed. B. Noninfectious girdle knot on the scion above a girdle of copper wire developed in the control bed. C. and D. Overgrowths on the scion following inoculations with a mixture of the grown-gall and hairy-root organisms.

time, it was desired to avoid complicating this with other physiological influences, including incompatibility, which doubtless may occur at the union on apple. It is well known, (12), (6) and (13), that an imperfect union may provide a girdling influence. This girdling influence at the union may result from several factors, including (1) poor fit, (2) poor wrapping,

TABLE 2.—The development of callus or wound overgrowths and noninfectious hairy root over two growing seasons on trees girdled with copper wire and planted in control beds and in beds containing crown-gall and hairy-root organisms, respectively

Organisms in soil	Trees wrapped with wire	Girdle knots	Noninfec- tious hairy root	Possible infec- tions ^a
	Number	Number	Number	Number
Results after growth during 1928 and 1929 in steamed soil				
Hairy root, No. 2014	34	16	10	4
Crown-gall and hairy-root mixture,				
No. 2004	37	15	15	4
Crown gall, No. 2018	38	14	12	1
Control	78	19	25	3
Results after growth during 1929 and 1930 in natural soil ^b				
Hairy root, No. 2014	244	107	30	3
Crown gall, No. 2018	143	15	7	3
Controle	161	11	13	0

^a It is uncertain from their appearance whether these overgrowths were due to the girdle or to the combined action of the girdle and stimulating organisms.

(3) failure of parts of the union to form callus, and (4) the formation of a cork barrier between the scion and root. These girdle knots were induced by the writers in 1927 during experiments aimed at control, in which the unions were wrapped with string treated with antisepties that prevented its decay. Hundreds of such knots were induced by different types of wrapping, which, due either to the nature of the material or to the method of application, did not decay before rapid secondary thickening of the apple stem took place. Although large girdle knots do not form in Wisconsin the first season from every tree so treated, those that survive this severe treatment over the second season nearly all show very considerable swelling and may even display masses of small roots coming from the region from $\frac{1}{4}$ to 1 in. above the constriction.

The character of these girdle knots induced by copper wire on the scion above the union is very similar to that earlier described (12) for wound overgrowths. A definite cortex surrounds the outer portion of the tissue after the knot has advanced beyond the fresh callus stage. This cortex may be quite irregular and show slight convolutions (Fig. 1, B). Not in-

b Dry weather retarded the development of these trees.

cAt the end of the growing season of 1929 25 trees bearing large overgrowths at the girdle were removed.

frequently these convolutions may contain various soil-inhabiting bacteria. The interior of these girdle knots is commonly very hard and may be composed of tissue that is laid down in irregular directions. making them exceedingly difficult to cut with a knife. While typical girdle knots are easily recognized, there are phases of the later development of infectious hairy root that superficially come very close to some of the reactions following girdles. In these border-line cases it is not possible as vet to distinguish between infectious hairy root and callus knot with full certainty in the absence of cultural examinations. Doubtless varying degrees of mixtures of these two overgrowths occur commonly in nature. The question is raised as to whether the infectious hairy-root development, itself, may act as a partial girdle, so that this influence may be operating to supplement the action of the bacteria. Noninfectious hairy root (Fig. 1, A) often appeared on the trees in these studies, especially during the second year. above the tissue formed by the copper-wire girdle (Table 2). They more frequently arose from the stem tissue from \(\frac{1}{2}\) to 1 in, above the point of constriction and resemble very closely the type of noninfectious hairy root that occurs commonly during some seasons on certain varieties of apple. such as Rome Beauty and Early Harvest.

The results from the experiments in both steamed and natural soil have been very consistent and have given so little mixture of the overgrowths studied that considerable confidence is placed in the outcome.

CALLUS NOT ORDINARILY AN OPEN INFECTION COURT

The manner of natural infection and its relation to callus tissue are the most critical considerations in the life histories of these organisms and may have very considerable importance in relation to the application of control measures. Working under ordinary conditions with the crown-gall organisms, Riker and Keitt (12) earlier reported that callus formed on apple grafts was "not commonly an open infection court" for the crown-gall organism. Rather similar results are reported by Siegler (15) for both his "peach" and "apple" strains.

The possibility of infection through callus tissue with several organisms was tried by the writers when the opportunity came to study this question under partially controlled conditions. A number of apple grafts were planted in different lots in beds treated, respectively, with the hairy-root organism, the crown-gall organism, and mixtures of these two, as well as in the control beds, as described earlier. Callus was induced upon the scions of young apple trees following wounds made during midseason. The wounds were produced by scraping the cortex with a scalpel until wood was exposed. These wounds were covered with soil and allowed to remain 3 weeks. After callus had formed certain of the trees in the con-

trol beds and beds to which bacteria were applied, respectively, were treated by covering the callus either with water or with cultures of the organism originally applied to the bed. Other trees were given the additional treatment of scraping the callus lightly with a needle after the water or bacteria, respectively, had been applied. Control inoculations with the same cultures through needle punctures produced positive reactions, which showed that the bacteria employed were in an active state. The results are shown in table 3. Here, it appears that a comparatively small percentage

TABLE 3.—Callus produced by injuries in midseason not ordinarily an open infection court for the crown-gall and hairy-root organisms²

Organism used in soil and	Trees	Reaction	s following trea	tments
for treatments	treated	Crown gall	Hairy root	Negative
	Number	Number	Number	Number
Callus covered with bacteria				
Hairy root, No. T43	27	0	0	27
Hairy root, No. 2014	31	0	1	30
Crown-gall and hairy-root				
mixture, No. 2004	28	1	0	27
Crown gall, No. 2018	27	0	0	27
Control	46	0	0	46
Callus covered with bacteria and scraped lightly				
Hairy root, No. T43	26	0	0	26
Hairy root, No. 2014	30	0	1	29
Crown-gall and hairy-root				
mixture, No. 2004	28	4b	1	23
Crown gall, No. 2018	27	1	0	26
Control	38	0	0	38

^a The cortex below ground was scraped, on August 7, 1928, until wood was exposed. Callus had formed by August 29, when it was treated by covering with bacteria or by covering with bacteria and then scraping lightly. The final results were recorded in November, 1929.

of infections were secured where the callus was not wounded, which indicates that under these conditions the callus is not ordinarily an open infection court. These results are in conformity with those reported earlier (12)

b Various degrees of hairy root were also shown by these overgrowths.

and (15). Even in the cases where the callus was lightly scraped the percentage of infection was very low. Since puncture inoculations of the woody stems (Table 1) gave a higher percentage of positive results, it appears that under these conditions callus tissue was not so susceptible to infection as the punctured stem tissues. The mechanism of callous infection needs further study.

The callus formed above wire girdles also seemed to be not commonly an open infection court for any of these organisms. This was tested in experiments in which the grafts were wrapped with wire, as earlier described, and different lots, respectively, were planted in various inoculated-soil and control beds (Table 2). Four times during the development of the girdle knots, mass cultures of the same strains of bacteria used, respectively, to inoculate the soil of the different beds, were employed to smear the surface of the callus on the girdle knots in these beds. At the end of the season the girdle knots (Fig. 1, B) that were produced in the different inoculated beds were alike in all cases except as noted (Table 2) and were like those produced in the control beds. In addition to the formation of girdle knots, a considerable number of root developments of noninfectious character (Fig. 1, A) were also found either at the top of the girdle-knot tissue or on the stem less than a half inch above the enlargement. These apparently were developed irrespective of any bacteria and seemed to be natural responses of the trees to the interrupted downward passage of metabolic products. Since the bacteria were placed in the soil where they persisted for a long time before the grafts were planted and since bacteria were subsequently added to the surface of these overgrowths at four different times without wounding during the course of their development, the presence of the bacteria in the soil and on the surfaces of these knots during the course of their development in certain soil beds seems beyond reasonable question. Although a few doubtful cases occurred, the relative uniformity in the appearance of these developments, grown both in soil containing different stimulating organisms and in soil containing none, suggests that callus tissue formed under such circumstances is not ordinarily an open infection court for these organisms.

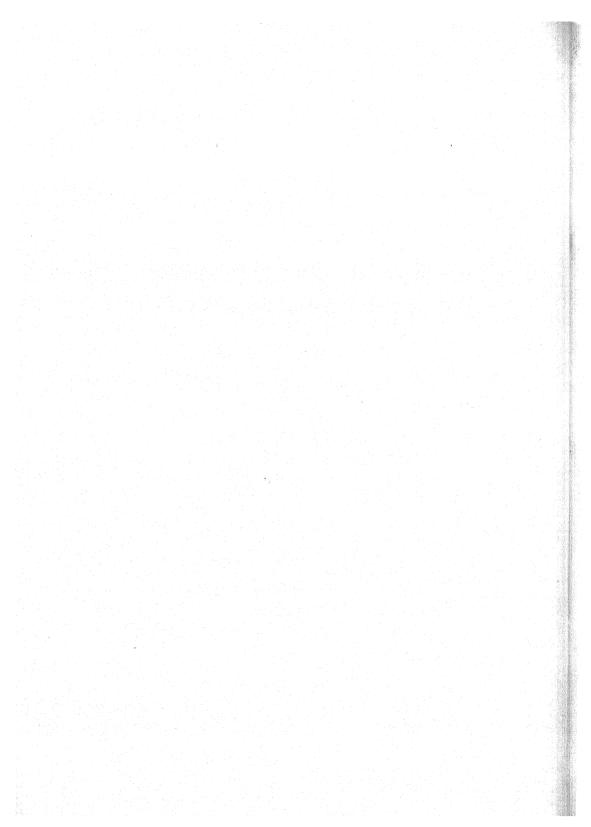
SUMMARY

- 1. Typical crown gall (*Phytomonas tumefaciens*), hairy root (*Ph. rhizogenes*), and girdle knots (copper wire), respectively, have been induced at will in both steamed and natural soil on nursery apple trees.
- 2. Inoculations with mixtures of the crown-gall and hairy-root organisms induced enlargements that showed both crown gall and hairy root, with a considerable range of the mixed characters.

3. Callus on Wealthy apple appears not ordinarily to be an open infection court for either of these bacteria.

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THE DEVELOPMENT OF CROWN GALL, HAIRY ROOT, AND WOUND OVERGROWTH IN GLASS CYLINDERS^{1, 2, 3}

A. J. RIKER, E. M. HILDEBRAND, AND S. S. IVANOFF

INTRODUCTION

The production of crown gall, hairy root, and callus knots or wound overgrowths on nursery apple trees has been attempted under conditions designed to prevent the interference of any stimulating factors aside from the one under consideration. The need for studies of this kind has been recognized for a number of years and was emphasized by Siegler (5). More or less controlled conditions have been employed by several writers, including Riker and Banfield (2), who have reviewed this situation. Although, in these reports, the evidence against a mixture of stimulating agencies is strong, the different writers recognize the possibility that uncontrolled factors might perhaps have complicated some of the results. For this reason the studies reported in the present paper were undertaken. It was planned to induce the various enlargements being considered under conditions in which the possible presence of confusing influences would be eliminated to the utmost with the available technique.

EARLIER STUDIES INVOLVING ROOT TISSUE

The attempt was made in the earlier stages of the work to enclose the unions of piece-root apple grafts in aseptic chambers. This was done because, in work previously reported (Riker and Banfield, 2), the objection was raised that undesired influences may perhaps have operated under the partially controlled conditions. The very exacting requirements necessary for growing even a herbaceous plant under aseptic conditions made it seem undesirable to attempt to grow young apple trees where all microorganisms were excluded. For this reason it was decided to work only with parts of young apple trees.

Large flasks of 3-liter capacity were employed as containers for growing piece-root apple grafts. In some cases these flasks were filled with sterilized white sand and nutrient solution and in others with steam-sterilized soil. The soil was autoclaved in small quantities for 5 hours at 15 pounds' pres-

- ¹ Published with the approval of the Director of the Wisconsin Agricultural Experiment Station.
- ² These studies have been conducted in cooperation between the Division of Horticultural Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, and the University of Wisconsin.
- 3 This work has been supported in part by a grant from the special research fund of the University of Wisconsin.

sure on each of 3 successive days. Aeration tubes to the top and bottom of the flask were provided through rubber corks. Piece-root apple grafts were prepared under aseptic conditions from surface-sterilized material and were inserted in these flasks with two buds extending through the rubber at the top. The seal between flasks, rubber stopper, graft, and glass tubes was closed by a layer of sterilized vaseline, 5 mm. deep. All the manipulations in these experiments were designed to exclude the entrance of microorganisms. For example, air filtered through sterile cotton was supplied as needed through the aeration tubes. The water employed to maintain moisture was distilled and sterilized. Inoculations were made on the piece-root grafts either at the time the experiment was set up or after the graft had become established by opening the chamber in a transfer room.

The results in the large flasks were rather disappointing for two reasons. First, under the conditions of the experiment, the piece-root apple grafts did not make sufficiently rapid growth to enable the various enlargements to develop in a manner parallel to that found in the field. Second, through a fundamental difficulty in the technique of surface sterilizing the roots from which the grafts were made, as explained later, the writers were unable to accomplish complete disinfection; consequently, the detailed results are omitted.

Glass cylinders were employed in parallel studies. These were fitted about the unions of piece-root apple grafts and planted in large containers, in the usual manner, in the greenhouse. In these earlier studies attention was directed particularly to the union between the scion and the root because this is the position at which the largest percentage of malformations occurs upon trees grown from piece-root apple grafts in the nursery. These cylinders are described in a later section of this paper. Grafts were prepared from surface-sterilized scions and roots and inserted in the sterilized glass cylinders under aseptic conditions. The tops and bottoms of the cylinders were closed with sterile sections of rubber stoppers and sterile vaseline. Waterproof rubber caps were provided over the ends of the cylinders before the grafts were planted in either large pots or soil cans in the greenhouse. In some cases the grafts on which the cylinders were mounted were planted so that the cylinders were beneath the soil; in others the cylinders were placed at the surface of the soil. The glass walls made possible frequent observations upon the developments that were taking place.

The results secured in the cylinders mounted on the unions of piece-root apple grafts were disappointing for the same reasons that have been given for the grafts grown in 3-liter flasks. The growth was not sufficiently vigorous to give results parallel to those secured in the field and it was not found possible to exclude all the undesired microorganisms. The greatest difficulty was experienced with various fungi that appeared from time to time

in many of the cylinders. Although the writers were not particularly concerned about the influence of these fungi upon the development of overgrowths, their appearance suggested that the technique employed was not sufficiently exacting, and results are not reported from such experiments.

The failure to secure aseptic conditions in the flasks and glass cylinders appeared to be primarily due to the difficulty in surface sterilizing the root stocks that were employed in making the grafts. This was due not to the inefficiency of the surface disinfectants employed but to the difficulty of reaching all the microorganisms without at the same time producing so much injury on the roots that they would not grow. The fundamental difficulty appeared following a closer examination of these seedling root stocks. It was evident that when the seedling roots were removed from the field a considerable number of very small rootlets were broken off during the process of pulling. Microorganisms entered the vascular tissue of these very small rootlets and penetrated the roots for a considerable distance. Any antiseptic, sufficiently strong to penetrate these tiny openings and kill the microorganisms present, was also strong enough to produce very severe injury to the root itself. For this reason such work involving seedling roots did not appear promising.

The details of the experiments with the cylinders placed about the unions are omitted because of these difficulties. However, these and subsequent experiments threw interesting light on the effect of adhesive tape on the development of callus from the cut surfaces and on the production of callus-like growth from the lenticels.

The effect of adhesive tape on the formation of callus at the union between scions and roots was observed in some of the experiments in these glass cylinders mounted about the unions of piece-root apple grafts. Where the union was only partially covered with adhesive tape, it was observed that an excess of callus tissue pushed out in some cases where the grafts were growing more or less vigorously, between the strips of adhesive tape (Fig. 1, A). This very strongly suggests that adhesive tape may act as a mechanical agency to prevent the lateral extension of excess callus and to force this callus tissue back through any openings that may occur between the cut surfaces of the graft. This appears to have the effect of preventing the formation of excess callus on the surface and of increasing the opportunity for good union between the scion and the root. This in itself would probably reduce the amount of girdling action due to a failure of union.

The formation of a considerable amount of tissue superficially resembling callus was also observed to appear from the lenticels of both the scions and the roots, which were enclosed in these glass cylinders and in which the humidity was maintained at a very high level. Küster (1) has discussed this subject. This type of development was rather confusing when the early

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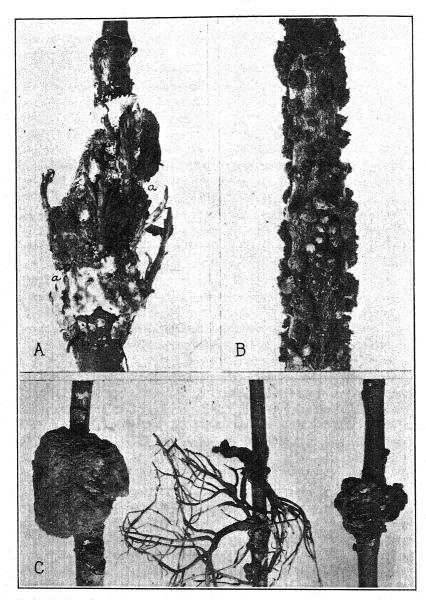


Fig. 1. Overgrowths on apple induced in glass cylinders. A. Excess callus induced at the union of a Wealthy apple graft that was only partially covered with ½-in.-wide adhesive tape. Depressions in the callus at a show the earlier positions of the adhesive tape. B. Developments from the lenticels of a Wealthy apple stem kept in saturated air. C. Maximum developments on the stems of Yellow Transparent apple trees following inoculations with the crown-gall organism and with the hairy-root organism and after girdling with copper wire, from left to right, respectively. The crown gall was shaped against the glass wall of the cylinder.

stages of the development of crown gall and hairy root were considered and presented the problem of satisfactory aeration within these chambers. An illustration of this type of growth from the lenticels of scion tissue may be seen in figure 1, B.

PRODUCTION OF OVERGROWTHS ON STEM TISSUE

Glass cylinders placed on the aboveground parts of nursery apple trees grown under natural conditions promised a means to avoid the difficulties that were encountered on seedling roots.

In the summer of 1928 and 1929 sterile glass or galvanized-iron chambers were employed. These chambers could be placed about portions of actively growing apple stems and so sealed as to allow the surface of the enclosed portion of the stem to be sterilized and to remain under aseptic conditions. This method is an adaptation of that employed by a number of plant pathologists to provide moist chambers about parts of plants. The cylinders used were usually 3 in. long by 11/4 to 11/2 in. in diameter. The cylinders were slipped over the tops of rapidly growing shoots of the current year's growth to the locations selected for special treatment. After being brought to position they were washed with a cloth wet with an antiseptic. The portion of the stem was prepared by removing all the leaves and also by washing it with an antiseptic, such as silver nitrate, 1 to 1,000. The lower ends of these cylinders were closed with comparatively thin sections of sterilized rubber stoppers of the desired thickness and diameter and with a tapering hole of suitable size in the center to permit expansion by growth of the stem without development of enough pressure to cause girdling. To insure perfect fit the rubber stopper at the lower end of the cylinder was covered in most cases with a layer of sterilized vaseline applied in a liquid state. For cylinders that were entirely closed, another stopper was similarly applied at the top. Aeration was supplied in some cases through sterile cotton in tubes extending through the upper stoppers. In some cases waxed-paper covers were also provided to prevent wetting by rain. monly, final sterilization was made by filling the entire cylinder with silver nitrate, 1 to 1,000, for 5 minutes. After the antiseptic was drained off, the inoculations were performed before the top was closed. Several variations of this technique were employed, which included filling some of the cylinders with soil that had been sterilized in small quantities for 5 hours on each of 3 successive days, at 15 pounds' pressure, and others of the cylinders with sphagnum that had been similarly sterilized. The glass cylinders were used almost exclusively in preference to similar cylinders made of galvanized iron because one could make observations more easily through the glass. A limited number of the cylinders were prepared with a lateral T extension, in in diameter by 1 in in length. This was closed with a rubber stopper

that could easily be removed to enable the operator to reach the stem inside without disturbing the upper or lower part. While this T extension was very useful for certain experiments it was found unnecessary for most of the trials, which did not require further attention after they were once started.

The efficiency of the method was tested by cultural examinations of the controls and overgrowths at the end of the experiment, which showed that many of them either were sterile of microorganisms or contained only the organisms introduced. A few of them became contaminated with fungi, but none except those inoculated showed any bacteria resembling in colony characters those in the crown-gall group. In these aseptic chambers placed about small portions of stems of apple trees, the various manifestations of crown gall, hairy root, and callus or wound overgrowth were induced without confusion of results due to undesired microorganisms. Although some difficulty was encountered in the 1929 and 1930 work, because of water of condensation in the chambers, this was practically overcome by providing satisfactory aeration through sterile cotton.

Crown gall was produced in these sterile chambers, which had the characters as described on apple by Riker and Keitt (4).

Hairy root, as described by Riker et al. (3), was likewise produced under these conditions, although the roots that developed following inoculation were frequently more fleshy, probably because of the relatively high humidity within the chambers. In many cases considerable root development was produced in cylinders that were exposed to full sunlight.

The callus, produced as the result of girdling, developed in a manner similar to that in the soil in the early stages. However, in the comparatively short time covered by the experiments with the sterile chambers in the 1929 and 1930 trials, the callus never reached the stage at which large woody knots were found. In some cases large soft callus knots were induced; in others, small hard callus knots were developed. The difference seemed to depend largely upon the relative humidity.

A summary of these 1928 and 1929 studies in aseptic chambers shows that negative results were secured in a number of cases because the interiors of the chambers were either too wet or too dry. Out of 70 trees inoculated with *Phytomonas tumefaciens* (Smith and Town.) Com. S. A. B., 16 showed well-developed crown galls and none showed hairy root. Out of 90 trees inoculated with *Ph. rhizogenes* R. B. W. K. and S., 43 showed well-developed hairy root and none showed crown gall. Out of 70 punctured control trees none showed any overgrowths, while of 20 girdled trees all showed more or less conspicuous girdle knots. In all the chambers with very high relative humidity there was a noticeable development of tissue from the lenticels (Fig. 1, B).

Repetition and extension of the experiments of 1928 and 1929 were carried out in 1930. Advantage was taken of the experience gained in earlier years, so that considerably better results were secured.

Several modifications of the methods employed deserve mention. experimental trees were 2 years old. The cylinders were usually placed on the lateral twigs that were produced the year before, which brought the cylinders several feet above the surface of the ground. The cylinders were closed both top and bottom with sections of rubber stoppers but were not sealed with vaseline. Chemical treatment of the surface was made for 15 minutes with a 20 per cent solution of a commercial sodium hypochlorite preparation (Bacilli Kill, containing 3.5 per cent sodium hypochlorite). When necessary, the surface of the apple stem was rubbed to remove air pockets. After this treatment the stem was washed with sterile distilled water for the same time. Inoculations were made with a scalpel at three points on the stem. The interior of the cylinder was then loosely filled with sterilized sphagnum that was moistened with a 5 per cent solution of the sodium hypochlorite preparation and closed with a section of rubber stopper. From time to time as it was needed, the sphagnum was moistened with additional quantities of this 5 per cent solution. Observations were easily made when desired by uncovering a hole in the upper section of rubber stopper, by inserting a sterile glass rod through this hole, and by pushing the sphagnum to one side. The concentration of the sodium hypochlorite solution was such that no noticeable injury was produced on the apple tissue, and no difficulty was experienced with contaminating bacteria or fungi.

The cultures employed in these studies were all the progeny of single-cell isolations. Crown-gall culture, T-5, and hairy-root culture, C-1, were employed. Their bacteriological characters have been given by Riker *et al.* (3).

A series of treatments were made on the stems enclosed in these cylinders during May, June, July, and August at Topeka, Kans. as shown in table 1. The stems were treated by wounding, by inoculating with the crown-gall organism, with the hairy-root organism, and with mixtures of both, and by girdling with copper wire. The responses made toward the end of the series were not sufficiently far advanced by the end of the growing season to be definite. This situation was probably due to the failure of the trees to continue growth. The drought of 1930 probably augmented this situation considerably. The detailed environmental conditions covering the period of this work will be reported in a later paper.

The incubation periods for the different reactions following various treatments, respectively, are given in table 1. The amount of radial extension necessary for accurate diagnosis varied with the different overgrowths.

The character of the reactions secured in this series of experiments is quite clear and typical (Fig. 1, C). Crown gall, like that described by

TABLE 1.—Incubation periods required for the development of various overgrowths in glass cylinders on the stems of Yellow Transparent apple trees following treatments at different times

Theothern	Total	Total	Minim	ım incuba	Minimum incubation periods following treatments on:	ls followin	g treatmen	its on:	
Treatments	treated	positive	May 26	June 9	May 26 June 9 June 23 July 7 July 21 Aug. 4	July 7	July 21	Aug. 4	
	Number	Number	Weeks	Weeks	Weeks	Weeks	Weeks	Weeks	
Woundsa	50	45	∞	7	9	9	80	q ¿	
Inoculations-									
Phytomonas tumefaciense	09	34	∞	7	8	∞	e	6°•	
Ph. rhizogenes ^d	09	34	8/12	4/10	5/10	÷/8	⊗•	<u>٠</u>	
Mixture ^e	30	18	8/12	7/10	6/10	\$/8	8>•	6 ~	
Wire girdlef	30	13	ıc	9	∞	∞ •	∞ •	&•	

a The time is that required for wounds to cork over.

b The interrogation points show that the typical response was not secured before November 15, the last day records were taken.

The time required for a 3-mm. radial extension of tissue and the formation of root primordia is expressed as the numerator. That required for roots over 6 mm. long is given as the The time is that required for a 4-mm. radial extension of gall tissue. d The single-cell culture, C-1, was employed. c The single-cell culture, T-5, was employed. denominator.

• A mixture of cultures T-5 and C-1 was employed. The time is given as for Phytomonas rhizogenes.

f The time is that required for a 2-mm. radial extension. Responses failed to develop in some cases, especially later in the season, because the stems stopped growing.

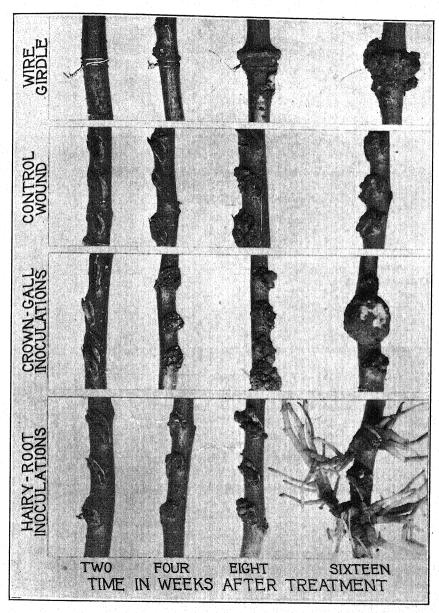


Fig. 2. Typical developments following various treatments at different periods of time on Yellow Transparent apple stems enclosed in glass cylinders.

Riker and Keitt (4), was induced following inoculations with *Phytomonas tumefaciens*. Hairy root, as described by Riker *et al.* (3), was induced following inoculations with *Ph. rhizogenes*. Inoculations with mixtures of these two showed various grades of mixed characters (Riker and Banfield, 2). The reactions to wire girdles showed callus knots or wound overgrowths that were quite characteristic (Riker and Banfield, 2). A summary of the inoculations made in May and June, 1930, which had satisfactory incubation periods are shown in table 2. The various stages in the de-

TABLE 2.—Development of various overgrowths in glass cylinders on the stems of Yellow Transparent apple trees

	Number	Stems showing number of					
Treatments	of stems treated	Only wound response	Crown gall	Hairy root	Mix- ture	Wire girdle	
Wounds	30	30	0	0	0	0	
Inoculationsa-							
Crown-gall organ-							
ism	30	6	24	0	0	0	
Hairy-root organ-							
ism	30	3	0	27	0	0	
Mixture of these							
organisms	15	1	0	0	14	0	
Wire girdle	15	0	0	0	0	13	

^a The responses to the crown-gall organism were counted only when the galls had a radial extension of over 4 mm. Those to the hairy-root organism had roots over 6 mm. long. Inoculations were made during May and June. The final results were taken November 15.

velopment of the more important types of reactions are shown in figure 2. Cultural examination of representative enlargements showed them to contain either no microorganisms or only those which had been inserted.

SUMMARY

These studies are interpreted as showing that crown gall, hairy root, and callus knot or wound overgrowth have distinct characteristics that may be induced by different agencies under rigidly controlled conditions. The methods employed have reduced to a minimum the possibility of a mixture of causal agents.

The best responses were secured following treatments made before the first of July.

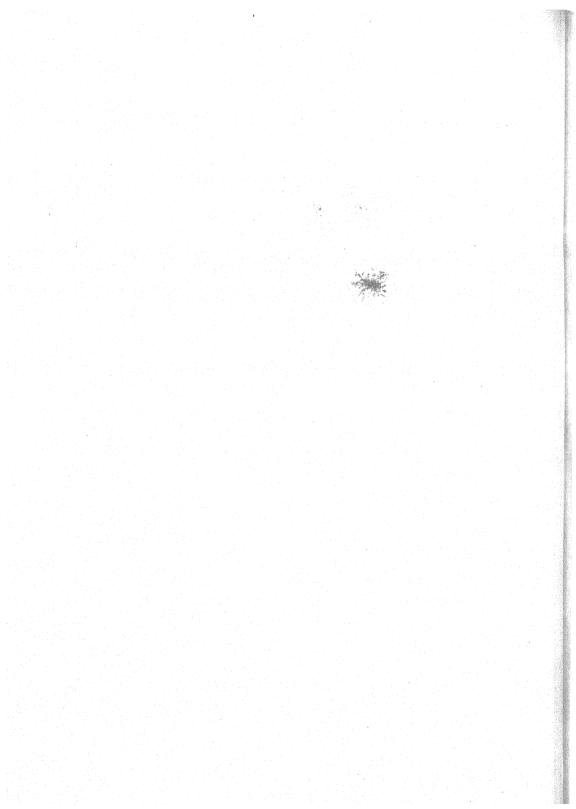
Incubation periods of at least 2 months were found desirable.

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THE PATHOGENICITY OF FUSARIUM CONGLUTINANS WR. AT LOW SOIL TEMPERATURES^{1, 2}

L. M. BLANK

For some years the idea has prevailed that 17° C. is the minimum soil temperature at which cabbage yellows becomes manifest in susceptible host material. This conception has arisen as a result of the work of Gilman,³ Tisdale,⁴ and Tims.⁵

In the studies reported by Gilman seedlings were grown in flats of infested soil in greenhouses where the temperatures were controlled only within wide limits. In several trials pots of seedlings were placed in glass chambers in an attempt to control the soil temperature more closely. Gilman concluded that soil temperatures of 12° to 16° C. prevented the occurrence of the disease and that characteristic symptoms occurred at temperatures of 17° or above. Exceptions to these conclusions, however, were noted occasionally at the lower temperatures.

Tisdale, using the Wisconsin soil-temperature tanks, studied somewhat more carefully the lower part of the temperature range of the fungus. His conclusions were in accord with those of Gilman, that "17° C. is approximately the low critical soil temperature for the disease occurrence." With highly susceptible strains of cabbage no disease occurred at soil temperatures of 14° and 15°.

Somewhat later Tims carried on studies with temperature-control equipment similar to that used by Tisdale. His results in relation to the occurrence of the disease at the lower part of the temperature range agreed with those of the previous workers. In no trial did the disease develop at soil temperatures as low as 15° C. and only a comparatively small percentage developed at 18°. Tims, however, does note that "the yellows disease is found in the roots of many plants grown at a soil temperature of 15° C."

In connection with other studies on cabbage yellows the writer has noted the disease to appear and progress in a destructive manner at what had been

- ¹ This study is the result of cooperative investigations between the Department of Plant Pathology, University of Wisconsin, and the Division of Horticultural Crops and Diseases, Bureau of Plant Industry.
- ² Published with the approval of the Director of the Wisconsin Agricultural Experiment Station and the Chief of the Bureau of Plant Industry.
- ³ Gilman, J. C. Cabbage yellows and the relation of temperature to its occurrence. Ann. Missouri Bot. Gard. 3: 25-84. 1916.
- 4 Tisdale, W. B. Influence of soil temperature and soil moisture upon the Fusarium disease in cabbage seedlings. Jour. Agr. Res. 24: 55-86. 1923.
- ⁵ Tims, E. C. The influence of soil temperature and soil moisture on the development of yellows in cabbage seedlings. Jour. Agr. Res. 33: 971-992. 1926.

considered the lower end of its temperature range. Therefore, experiments were initiated to determine more clearly the minimum soil temperature for the development of yellows in cabbage and other subspecies of *Brassica oleracea* L.

The experiments were conducted during the months of December and January for it was necessary that the soil temperatures be controlled as closely as possible. The trials were therefore carried on only during these months for three winters. The seedlings used in the several experiments were grown in clean soil for a month or more in the greenhouse at temperatures ranging from 15° to 20° C. The soil into which the seedlings were transplanted had been inoculated several years previously with cultures of the yellows organism and was known to be thoroughly infested. The cans containing these seedlings were immediately placed in the Wisconsin soiltemperature tanks and the desired temperatures attained by running a continuous stream of cold water into the tanks, or by use of an electric heating unit, if higher temperatures were desired. The air temperature in the greenhouse varied around 15° C., without excessive increases during the middle of the day. To further control the soil temperature in the cans about an inch and a half of ground cork was placed on the surface of the soil following transplantation of the seedlings. Standardized thermometers were placed in the cans of soil and in the water in each tank. The soil moisture in the cans of seedlings was maintained at a condition favorable for seedling development. The seedlings used in the several trials were of commercial lots and included three varieties of cabbage, three of kale, and one each of collard, cauliflower, kohl-rabi, and Brussels sprouts. The seed lots did not include any of the yellows-resistant selections but represented commercial varieties in common use. The varieties used in the trials were Moss Curled Dwarf, Thousand-headed, and Siberian kale; New Snowball cauliflower; Georgia collard; White Vienna kohl-rabi; Long Island Improved Brussels sprouts; and Early Jersey Wakefield, Early Flat Dutch, and Glory of Enkhuizen cabbage.

The preliminary trials were conducted mainly with subspecies of *Brassica oleracea* other than cabbage. In trial 1 the three kale varieties were studied at 2-degree intervals of soil temperature, the range being from 12° to 20° C. Previous workers⁶ have noted the difference in susceptibility to yellows displayed by these three kale varieties, Moss Curled Dwarf being the most susceptible and Siberian kale, the least. In the temperature tanks similar results were noted. The yellows was evident in the Moss Curled Dwarf at 14° and above, with the disease severe enough to result in dead

⁶ Walker, J. C., and F. L. Wellman. A survey of the resistance of sub-species of *Brassica oleracea* to yellows (Fusarium conglutinans). Jour. Agr. Res. 37: 233-241. 1928.

seedlings at 16°. Thousand-headed kale seedlings showed symptoms of the disease at 16° and dead plants were present in the 18° C. tank. No disease was evident in the Siberian kale seedlings at 21° C., the highest temperature studied. Approximately 45 seedlings of each variety were examined at each of the temperatures.

The next test was made the following winter and included seedlings of Moss Curled Dwarf and Thousand-headed kale, kohl-rabi, and cauliflower. The temperatures at which the seedling reaction was studied were 14°, 16°, 17°, and 19° C. After 33 days' exposure to these temperatures the final notes were recorded and it was found that the disease symptoms were manifest in both kale varieties and in kohl-rabi at 14° and in the cauliflower seedlings at 16°. Seedlings killed by the disease were present in the 16° tank in all lots except cauliflower, where they first appeared in the 19° tank. In this trial approximately 24 seedlings of each lot were considered at each of the 4 soil temperatures.

The evidence gained in the foregoing trials indicated rather conclusively that the minimum temperature for the occurrence of the yellows was below 17° C. Therefore in another final trial the soil temperatures were controlled with extreme care. Two of the tanks were regulated to give a soil temperature of 12° to 13°, 2 at 14° to 15°, and 1 at 16° to 17°. The soil temperature in the tanks was further controlled by the use of about $1\frac{1}{2}$ in. of ground cork, placed on the surface of the soil in the cans following transplantation of the seedlings. Thermometers, inserted into the soil about 14 in., were read several times daily and the temperatures recorded. Over the 42 days' duration of the experiment 92 soil-temperature readings were recorded for each tank and the average temperature prevailing in each tank was computed from these readings. The maximum soil temperatures recorded in the various tanks were 14°, 14.3°, 15.8°, 14.8°, and 17.3° C., respectively. In only one case did the maximum soil temperature exceed the point previously considered as the lower critical temperature for the occurrence of the disease. We may consider the four lowest temperature tanks as having an average soil temperature well below 17° C., as indicated in the table, and also with a maximum temperature below the previously reported critical temperature.

Varieties used included three of cabbage, and one each of kale, cauliflower, collard, kohl-rabi, and Brussels sprouts. The seedlings, which had been grown in yellows-free soil, at an air temperature of about 15° C., were transplanted when 34 days old into cans of yellows-infested soil and immediately placed in the tanks. Thirteen seedlings of each lot were studied in each of the temperature tanks. The results of this test are given in table 1.

The symptoms of the disease appeared in the collard seedlings in tank V 12 days after transplanting them into the yellows-infested soil. After 18

TABLE 1,-0 courrence of yellows in crucifer seedlings exposed for 42 days at various soil temperatures

		11. 11. 11. 11.					
	Total	diseased	ro	10	32	29	99
		Glory of Enk- huizen	0	63	9	5	6
	Cabbage	Early Flat Dutch	63	67	7	6	10
sed		Jersey Wake- field			9	9	12
Number of seedlings diseased		Kohl- rabi	0	Ħ	-	-	7
umber of see		Kale	2	c 3	ന	c ⁄1	7
Ä		Brussels sprouts	0	0	H	¢1	2
		Collard	0	Н	41	က	10
		Cauli- flower	0	H .	4	Н	4
	Average	soil temperature	∘C. 12.1	12.4	14.4	14.6	16.5
	Tank	No.	H	II	H	IV	Δ

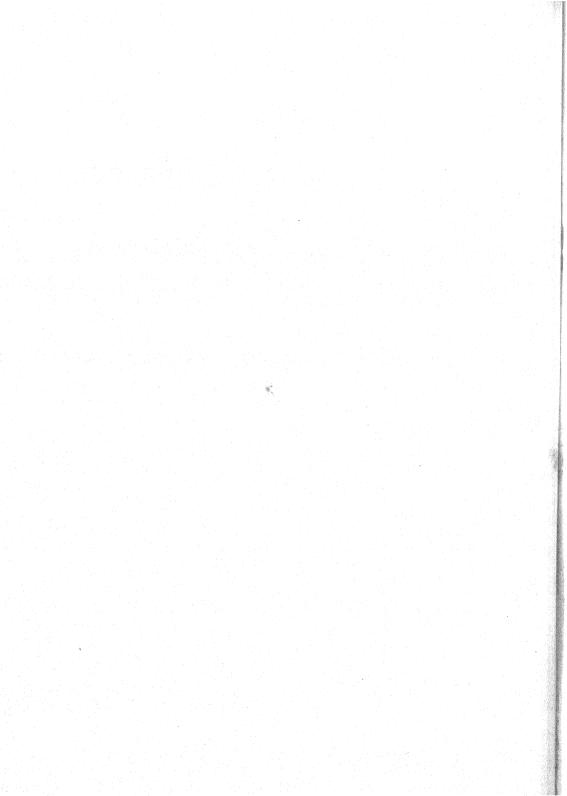
a Thirteen seedlings of each lot at each temperature.

days it was evident in seedlings of the remaining lots in the tank; at the next lower temperatures (tanks III and IV), the disease was first noted after 20 days and in tanks I and II after 25 days. At the lowest temperatures (tanks I and II), the disease was manifest in 5 and 10 of the total number of seedlings, respectively, after 42 days, as shown in the above table. The disease symptoms at these lowest temperatures were slight, usually evident on but one leaf per plant. In tanks I and II the organism was recovered from kale and from two varieties of cabbage; in tanks III, IV, and V it was isolated from each of the 8 lots of seedlings.

The amount and severity of the disease were directly dependent on the soil temperature, as has been noted by the previous workers. It may be observed that the amount of disease in tank IV was slightly less than that in tank III, although the average soil temperature was slightly higher. This slight discrepancy, however, may be explained by the fact that the maximum temperature recorded in tank III was higher than that of tank IV and that, although the average temperature was lower, the higher temperature, prevalent in tank III for a few hours, may have been sufficient to stimulate the development of the disease in tank III. The severity of the disease was sufficient in tank V to result in the death of seedlings of all varieties, and, in some lots, to the extent of half or more of the total number of seedlings. An occasional seedling was killed by the yellows organism in tanks III and IV, while at the lowest temperatures (tanks I and II) the seedlings that showed symptoms of the disease were not killed.

From the foregoing results it is concluded that the yellows of crucifers may develop at a temperature considerably below that previously reported. Since the disease was apparent at the lowest soil temperature studied in the present trial the low critical temperature for the development of the disease has not been determined, but it is thought to be close to 12° C. The writer, however, is convinced that cabbage yellows may become manifest at a soil temperature of 12° to 13° C., which is considerably lower than the heretofore-reported soil temperature of 17°.

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BOOK REVIEW

Miller, Edwin C. Plant Physiology. vii-xxiv + 900 pp., 38 figs. Edition I, McGraw-Hill Book Company, Inc., New York and London. 1931. Price, \$7.00.

Here at last we have what is probably the most comprehensive treatise on plant physiology in the English language. A work of this character, treating the subject as it does from the standpoint of the basic importance of the plant cell; of solutions and membranes in relation to the plant cell; the intimate and complex relation of the plant and its various organs to their environment; the various vital processes peculiar to the growing plant, such as photosynthesis, processes of digestion, translocation of materials, respiration, and growth, will at once prove a valuable adjunct to not only the working library of the plant physiologist but also that of the plant pathologist, the agronomist, and the horticulturist.

The author's long service as a teacher and his extensive experience in botanical research has given him an unusual opportunity to acquaint himself with the now voluminous literature on the subject. This familiarity with his field and the published matter bearing upon it is plainly evident to him who takes the time to thumb leisurely the 850 pages of meaty text.

That ecologist or plant pathologist or other plant-science student, be he teacher or investigator, cannot review but with profit to his teaching or his research the first and second chapters of this text on plant physiology. It will, no doubt, seem to some that the author has devoted unnecessary space to a more or less elementary presentation of definitions and facts long known and not in the strict sense of the word a part of the lingo or thought of plant physiology. Yet, we must admit that an understanding of the plant cell—its structure as well as its behavior—is fundamental to our grasp and interpretation of facts disclosed by our research on the normal and abnormal physiologic behavior of plants.

On reading the text of Miller's Plant Physiology some may experience a sense of disappointment on finding that too little of it is substantially Miller's. One should not, however, overlook the fact that the evaluation and interpretation of the scientific worth of the more than 3,150 papers referred to in the text are Miller's. Moreover, it is probable that hundreds of papers not referred to at all were subject to his judgment.

One reader and critic of the work has said of it: "It is a remarkable treatise on the subject of plant physiology; remarkable for what it contains and for what it omits, and notable among the things omitted is the author's own contribution to the subject." Evidently, our critic had not taken cognizance of the fact that there are here and there throughout the text as many as 58 references to work done and published by Doctor Miller,

himself, since 1910. The author's most important contribution to plant physiology, quantitatively and probably qualitatively, has come through his investigation of the duty of water in plant metabolism and the transpiration phenomena of various crop plants. And yet, notwithstanding this fact, he has not devoted a disproportionate amount of space to the chapters bearing directly on these aspects of the subject in hand.

The treatment of the following chapters: The Roots of Plants; The Intake of Water by Plants; The Elements Absorbed by the Plant; The Loss of Water from Plants, and The Translocation of Materials in Plants could have been improved had the author devoted more space to the consideration of factors, vital and other, that inhibit the proper functioning of plants and plant organs. On the other hand, there is sufficient reference to such factors as soil-infesting and plant-disease organisms to indicate to the plant pathologist and the agronomist the unmistakable importance, if not necessity, of their acquiring a more thorough working knowledge of the relationship of such organisms to crop ecology.

We need to know much more than we do about the relation of root-invading organisms to root development of the host, their effect on the metabolism and water requirements of the host, their effect on the water-intake capacity of the invaded plants, and their ability to produce fruit or seed. We need to know more than is known about the effect of plant rusts, mildews, smuts, and the more obscure parasitic organisms on the quality as well as quantity of fruit or seed. As an important aid to such research we have Miller's Plant Physiology pointing the way, replete with references to a voluminous literature the digestion of which cannot but prove invaluable to those engaging in crop-ecological or phytopathological research.

The author's comprehensive review of the literature on the intake of water by plants, in which he devotes special attention to (1) sources of the plant's water supply, (2) wilting coefficient and factors influencing it, (3) water-supplying power of the soil and time of wilting, and (4) entrance of water into the root, should prove helpful to the student of root and foot rots of various crop plants and to those engaged in the investigation of the relation of rusts and other parasitic fungi to the water requirements and total yield of plants.

The author's thoroughgoing treatment of the subject matter of Chapters VIII, IX, and X, entitled The Formation of Carbohydrates by the Green Plant, The Nitrogen Metabolism of the Green Plant, and The Fat Metabolism of the Green Plant, cannot but prove helpful to teacher and investigator alike. We have long needed just this sort of valuable service by one who is at once the accomplished, exacting teacher and research worker in plant physiology.

It is all very well for the plant pathologist to inform himself on the details of the physiology of parasitism, the various steps and stages in the life history of his smuts and rusts and mildews and root-rot organisms, the symptoms and etiology of this or that plant disease, but it is quite as fundamentally important that he fully acquaint himself with the structure, growth, and physiologic behavior of the disease-free host. This done, he can the more intelligently interpret the results following invasion of the host by this or that parasitic organism or the abnormal behavior of plants when subjected to conditions of mineral deficiency or meteorologic upset.

The host of this or that pathogen is, for the period of active parasitism of the pathogen, its medium. But the medium, unlike potato agar or some other kind of agar or gel, is a vital thing played upon by light, heat, humidity, salts in solution, and a variety of other factors. It receives, digests, assimilates, elaborates, grows, breaks down under catalytic agents, and reproduces its kind. All of these processes are ably presented to the reader by our author and thus made available to the investigator in the fields of plant physiology and phytopathology. One can read between and within the printed lines of these chapters the fact that the ultimate expression of the parasitic consequences of fungous invasion or bacterial infection must of necessity depend upon the status of the medium. The medium is sensitive, unstable, today one thing, tomorrow another. All this is clearly and effectively brought out by our author.

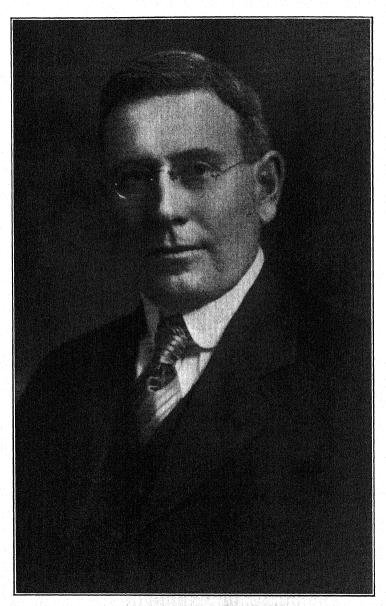
In his treatment of the subject matter of Chapter XI, "The Process of Digestion in the Green Plant," the author points out the fact that the food requirements of the protoplasm of plants and animals are alike. His account of enzymes and the rôle of enzymes in the digestion of plant-food materials is a comprehensive summary of the subject as it has been developed through the research of scores of plant physiologists and biochemists throughout the world. Here, again, is subject matter of great importance to the phytopathologist and the agronomist. Often, when seed of corn, wheat, flax, or other plant is sown or planted, there arise many abnormal plants and not infrequently there is considerable seedling mortality from one cause or another. Possibly the seed is inherently weak, poor in the enzymes necessary to digestion. More often, however, the lack of seedling vitality is traceable to conditions inimical to catalytic action or favorable to fungous invasion and the consequent consumption of the food materials stored up in the seed or other plant structure.

Chapters XII and XIII, entitled "The Translocation of Materials in Plants" and "The Process of Respiration in Plants," respectively, should prove helpful to those engaged in agronomic and plant-pathologic research. It is obvious that a more exhaustive knowledge of the phenomena discussed in these two chapters might dissipate much of the mystery and mental fog

that make difficult and slow our progress in studies on winter hardiness of alfalfa, wheat, and other crop plants. Such knowledge might facilitate our progress in the investigation of such obscure physiologic derangements as straighthead of rice, internal tissue degeneracies, premature decline of vitality and loss of germinability in seeds of various plants, spindle tuber of the potato, floret sterility in many of our crop plants, phloem necrosis, storage rots, etc. Likewise, the 14th and final chapter of this important work contains much that cannot be other than helpful to the student of growth phenomena and the factors that promote or inhibit growth.

The reader of this work on plant physiology will undoubtedly be impressed by the fact that it is first of all what will surely prove to be a useful compilation of information on the various functions of the growing plant and the factors affecting one way or another the plant as a living entity. If he employes it in the classroom he will find it far more useful as a reference book than as a laboratory or classroom text. Its usefulness as a reference book could have been increased had the 36 pages, devoted to more or less orthodox test or examination questions, been filled with pertinent and needed illustrations of apparatus, graphs, and other matter supplementary to the text. Another and revised edition should take cognizance of such desirable improvements and should be given the benefit of more detailed attention to matters of grammatical construction and clarity and comprehensiveness of table legends.—H. B. Humphrey, Bureau of Plant Industry, U. S. Department of Agriculture, Washington, D. C.





FREDERICK JOHN PRITCHARD

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FREDERICK JOHN PRITCHARD 1874–1931

WILLIAM S. PORTEL

The life of Frederick J. Pritchard was full of energetic and enthusiastic activity until the very hour of his demise. Endowed with a keen intellect and a powerful physique, verily he was a human dynamo and his interests in human affairs were legion. During the more than 12 years of close association with him, the writer never saw him relax but momentarily. To the incessant urge within him that overtaxed his heart, his friends attribute his untimely death, which occurred while seated at his desk in the Bureau of Plant Industry in Washington, D. C., January 13, 1931. At the time of his death, Mr. Pritchard was Senior Physiologist, Division of Horticultural Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture.

Born in Camanche, Iowa, on December 24, 1874, of sturdy Scotch-Irish stock, Fred Pritchard spent his boyhood years on a farm northeast of Council Bluffs, Iowa, where he had an excellent opportunity not only to develop into robust manhood but also to study at first-hand the mysteries of life as nature unfolded them. His analytical mind early pried into the secrets of wild life about him. In his mature boyhood he learned the fundamentals of zoology through the help of his father, who was a very successful veterinary surgeon. During all this time his noble mother was quietly instilling in her son high ideals and worthy ambitions, which became so ingrafted into his very nature that throughout his life Fred Pritchard never lost sight of those precepts and frequently referred to them. He attended grammar school at Persia, Iowa, walking 6 miles each day.

In early manhood he obtained a thorough knowledge of farming operations and the growing of farm crops by actual experience on the farm. As soon as he had mastered practical farming his thoughts turned to enlarged fields of learning. With money he had earned farming, he attended the Woodbine Normal School, Woodbine, Iowa, and as he advanced in his school work he took up teaching to earn his way through school. He then went to Omaha, Nebraska, and earned his way through business college, working

¹ The writer gratefully acknowledges his indebtedness to Dr. H. A. Edson, Chief Examiner, Civil Service Commission, to Dr. A. G. Johnson, Principal Pathologist, Bureau of Plant Industry, and to Dr. W. T. Pritchard of North Platte, Nebraska, for valuable suggestions and criticisms made in the preparation of this sketch.

evenings and vacations as clerk in a freight depot. With an alert mind and a sturdy body as his resources, he matriculated at the University of Nebraska in 1900, and majored in botany, plant physiology, and plant pathology, graduating 4 years later with the high esteem of the faculty. After graduation he continued for a short period as a graduate student at his alma mater and also served as assistant in botany. After this he accepted the position of instructor in botany at the North Dakota Agricultural College, later becoming assistant professor of botany, which position he held until 1907. There he became deeply enthused over the possibilities of breeding varieties of crop plants that would resist diseases. He resigned his position at the North Dakota Agricultural College to pursue graduate work in plant breeding and plant physiology at Cornell University. After 2 years he transferred to the University of Wisconsin, where he completed the residence requirements for the degree of Doctor of Philosophy in 1910.

It was while at Wisconsin that Mr. Pritchard, as Agent in the United States Department of Agriculture, became interested in breeding sugar beets. In 1911 he was appointed Assistant Physiologist and took charge of the sugar-beet-breeding work in the Bureau of Plant Industry. Extensive studies had been undertaken and much progress made in developing strains of sugar beets that yielded high percentages of sugar when it became necessary to terminate this work in 1914. Only those familiar with plant breeding can appreciate the immense amount of painstaking work involved in carrying out the preliminary phases of such a piece of research and the disappointment of the breeder when forced to abandon it before its completion. However, with undaunted courage, Mr. Pritchard embarked on a new field of endeavor, namely, the breeding of disease-resistant tomato varieties, in which he later attained the crowning success of his career.

Although the funds available were very meager, with characteristic enthusiasm and foresight he planned the work on such a large scale that some tangible results were obtained in a relatively brief time. He also laid the groundwork for a long-time tomato-breeding program. He quickly assembled varieties and strains of tomatoes from all parts of the world and tested them for resistance to the Fusarium wilt disease that was causing heavy losses in many important tomato-producing centers. Fortune surely smiled on this work, for within 4 years Mr. Pritchard presented to the tomato industry the Norton, Columbia, and Marvel varieties, which were wilt-resistant selections made, respectively, from the Stone, Greater Baltimore, and Marvel of the Market varieties. In the meantime, Mr. Pritchard had been selecting the most promising individual plants from a number of tomato varieties and crossing them. It was from this material that the Marglobe variety finally was developed. Probably no other tomato variety

ever has received a more spontaneous and universal reception by the industry than this variety.

Back in 1918, Mr. Pritchard was testing his wilt-resistant tomato hybrids for resistance to tomato blights of various kinds. In carrying out this phase of the work he showed unusual foresight. In 1924 the Department received an emergency call for assistance in saving the winter-tomato industry from the ravages of the nailhead-rust tomato fruit spot. The resistance of the Marglobe variety to this disease was so marked that the commercial damage from it proved negligible. It quickly became not only one of the leading shipping tomatoes but also the principal canning variety in many of the large canning centers.

Besides originating several other valuable tomato varieties, Mr. Pritchard made important contributions to the subject of plant-disease control by field sanitation methods and also introduced new copper combinations as fungicides. He contributed to our knowledge of the effect of soil fertility and cultural conditions upon the development of certain plant diseases and also worked out the life cycles of several fungi that are parasitic on tomato plants. In addition, he started work on breeding disease-resistant cucumbers and eggplants.

Mr. Pritchard possessed an unusual ability for making friends quickly. He had a personal magnetism that appealed to those whom he met, and he immediately won their respect and confidence. Managers of large agricultural enterprises who knew him eagerly sought his advice and counsel, not only on the subjects in which he specialized but also regarding matters in unrelated fields. Their confidence in him seemed to be implicit. On several occasions captains of industry who had met him have spoken to the writer regarding Mr. Pritchard's pleasing personality, his modesty, and his great store of accurate information on a wide range of subjects. He was very widely and favorably known by practical agriculturists all over the United States, and he also had much correspondence with agriculturists in foreign countries.

Only a person with extraordinary energy could have maintained such an active interest in so wide a range of subjects as did Mr. Pritchard. Repeatedly he would maintain intensive concentration on a pathological or breeding problem for weeks at a time and at the same time have some theoretical problem in an unrelated scientific field as his chief diversion, or hobby.

Fred Pritchard was a man of dauntless courage and endurance and did not hesitate to assume a solitary position on any subject when he considered his contemporaries were championing error. When he embraced an idea he never gave it up unless he had convinced himself of its fallacy, even though he stood alone against the field. Through the mellowing years he evolved a philosophy of life that was at once optimistic and hopeful. Although he was an indefatigable worker and allowed himself practically no relaxation, he contended that mankind should have more time for leisure in order better to enjoy the beauties of nature and life; also that intolerance of the views held by others is engendered by the slavish grind that we impose on ourselves, which, in turn, causes much of the misery and irritation of our modern civilization.

Despite Fred Pritchard's penchant for ceaseless work, he was devoted to his home and family and spent every minute he could at home. This is the reason why he was rarely seen at public meetings except when duty called him. The loss of his young son Robert in 1926 was a shock from which he never fully recovered. However, he never complained and he bore his grief in silence. He is survived by his wife, Selma I. Pritchard, and only daughter, Dorothy.

Mr. Pritchard was a member of the American Association for the Advancement of Science; the Washington Academy of Sciences; the American Genetics Association; The American Phytopathological Society; the Botanical Society of Washington; Alpha Zeta, and Sigma Xi.

BUREAU OF PLANT INDUSTRY,

U. S. DEPARTMENT OF AGRICULTURE.

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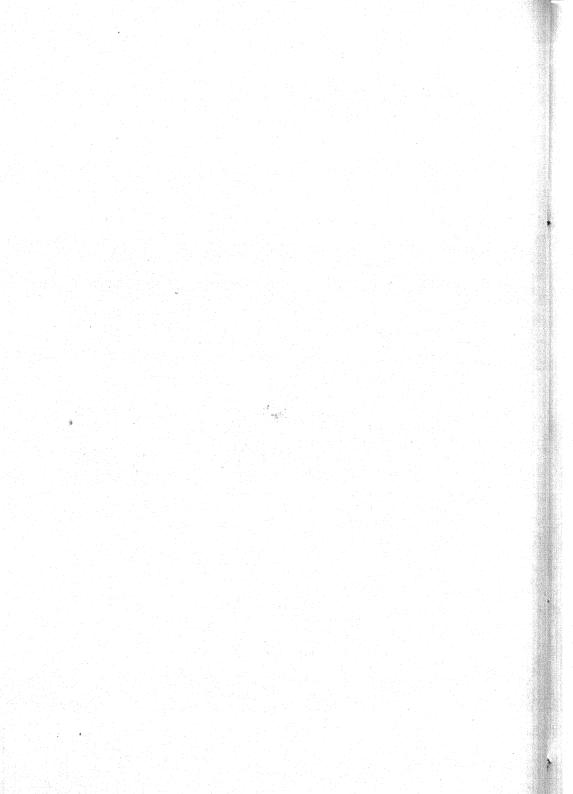
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A PRELIMINARY REPORT ON RESISTANCE TO CURLY TOP OF SUGAR BEET IN BEAN HYBRIDS AND VARIETIES

W. W. MACKIE AND KATHERINE ESAIT

Curly top of sugar beet (Fig. 1) has been known to attack beans since 1919, when Carsner (1) reported its appearance in Idaho. Since then Severin, according to Carsner (3), has found that a large number of varieties of beans are injured by curly top of sugar beet. He noted especially that the plowing under of sugar-beet fields caused the beet leaf hopper,



Fig. 1. Varieties of sugar beets under test for resistance to curly top. Center 4 rows, susceptible variety. Varieties to the right and left, resistant.

Adjacent to the bean plots.

Eutettix tenellus (Baker), to migrate to fields of beans that later developed severe injury from curly top. Severin and Henderson (5) found that naturally infected varieties of beans included Small White, Cranberry, Kentucky Wonder, and Long Red Kidney, of the species Phaseolus vulgaris, and Henderson Bush or Baby Lima (P. lunatus sieva). In addition, he experimentally infected with curly top the following varieties: Bayo, Blue Pod, Lady Washington, Pink, Red Mexican, and Spotted Red Mexican, of the P. vulgaris group, Scarlet Runner (P. multiflorus), and Burpee's Bush, Fordhook, and Lewis Limas (P. lunatus). Severin observed that the Lima beans showed in field and greenhouse much less injury from curly top than common beans (P. vulgaris), Scarlet Runner, and Blackeyes (Vigna sinensis).

The California Pink bean, long noted for its hardiness and high productivity, in California, was believed by Carsner (1, 2) in his first observations to be nonsusceptible to curly top. Later, Severin (4, 5) was able to induce curly-top symptoms by artificial inoculation in the greenhouse with infective beet leaf hoppers. Many California Pink bean plants failed to show symptoms of the disease, indicating, he believed, that there are races of highly resistant or immune California Pink beans.

Several years ago the senior writer secured a cross between a strain of California Pink and the variety Robust, a white pea bean, resistant to mosaic. Hybrids from this cross in the 8th generation and fixed for color, maturity, vine habit, and the commonly desirable characters of field beans were tested for their resistance to curly top. To this list was added Hopi, Baby Limas, and Butter beans. The varieties were planted in very short rows, 30 in. apart, in order to make certain that an abundance of infective leaf hoppers would attack each vine. Nymphs instead of adults were used in order to confine the insects to the actual vine upon which they were placed (Fig. 2). The rearing of the infective beet leaf hoppers, placing

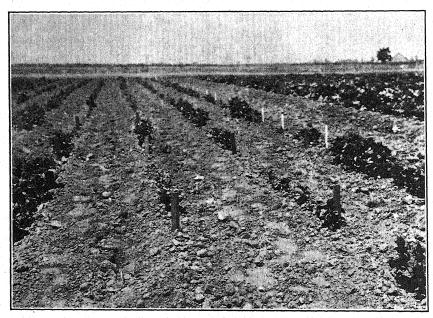


Fig. 2. Bean hybrids and varieties under test for resistance to curly top. Sugar-beet hybrids under similar tests to the right.

them on the bean vines, and the taking of careful notes and observations on the resistance of the beans to curly top were the work solely of the junior writer, who has done considerable research on curly top of sugar beet. The

TABLE 1.—Resistance to curly top in bean hybrids and varieties, 1930; planted May 13, 1930—Davis, California; inoculated with in-

D	Variety	Identifica- tion	Seed	Number of plants	Grades of resistance	Number of plants with		Bearing plants	Resistance Class
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32		10(59)27	M	24		н	G	4	c

TABLE 1.—(Continued)

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TABLE 1.—(Continued)

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results of the tests with 96 hybrids and varieties of beans are presented in table 1.

DISCUSSION OF RESULTS IN TABLE 1

Correlation of seed-coat color with resistance. Red seed-coat color in beans is supposed by many to be correlated with resistance to curly top, and white seed coat, with susceptibility. This observation is furthered by the very high resistance of the common pink bean and other red and pink varieties. The actual tests at Davis, during 1930, showed the California Pink free from curly-top injury under heavy infection, but several red and pink varieties, including Red Kidney and China Red, were destroyed by curly top. Brown Bayo was also destroyed, and mottled and striped colored beans, including the Pinto, Nagazura, and Striped Kidney, were similarly injured. The mottled Cranberry, however, was found to be highly resistant.

Among the Robust × Pink hybrids the correlation between susceptibility and the white color did not hold, for a number of white hybrids were found highly resistant to or free from curly top. Conversely, some of the pink hybrids were destroyed by curly top. A greater proportion of the pink hybrids were resistant to curly top than were the white hybrids.

Grades of susceptibility. Five grades indicating the severity of the curly-top attack were used: Grade 1 (Fig. 6) includes those beans that show little or no symptoms of curly top and no apparent reduction in yield. Grade 2 (Fig. 3) indicates a visible but very slight curly-top infection; grade 3 indicates susceptibility; grade 4 includes highly susceptible plants;

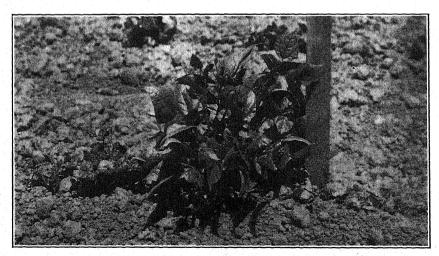


Fig. 3. Robust×Pink hybrid (white). Heterozygous susceptibility. Grades 5 to 1—left to right.

and grade 5 includes those plants killed or rendered entirely unproductive by the disease.

The effect of curly-top infection is greatest when the bean seedlings are entering the 3rd- and 4th-leaf stage. When the initial infection occurs later the injury is correspondingly less. Mature plants show but little injury when subject to first infection at this stage. Certain varieties possessing a degree of resistance show marked ability to recover after the first injuries following curly-top infection (Fig. 4). In some cases the older branches

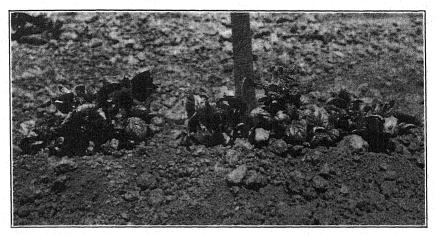


Fig. 4. Robust \times Pink hybrid (white). Very susceptible. Grade 4. No beans produced.

on such resistant plants show pronounced symptoms of curly top, whereas the later growth does not indicate the presence of the disease. These differences were noted in a change of grade when observations taken July 1 and July 23 were compared. The reverse, or increasing intensity of attack, was not found.

Curly top distinguished from mosaic. The virus diseases attacking beans in California, curly top and mosaic, are common and wide-spread. Both are carried by insects, Eutettix tenellus (Baker) and Aphis rumicis, respectively, but the latter is known to be carried in the seed, which has not been observed in the case of the former. These diseases may be distinguished in the field by certain characteristics. The bean mosaic, more particularly, affects the young leaves. They are reduced in size and are cupped downwards. When held to the light areas of irregular size, both lighter green and darker green than the normal green are seen. Cupped or raised areas of dark green may be seen on the upper surface, and the smaller veins on young leaves do not appear transparent, as they do in the case of curly top. The curly-top-infected leaves do not show any mottling but are darker in color than normal and develop a puckering and an out-

ward cupping of the young leaves (Fig. 4). Both diseases cause a stunting of the plants. The small veins appear plainly clear or transparent. In the Robust × Pink hybrids there is positive indication that a satisfactory variety resistant to both diseases can be secured, for the Robust parent (Fig. 5) is resistant to mosaic and the Pink parent to curly top (Fig. 6).

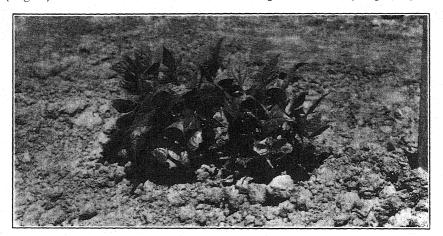


Fig. 5. Robust (white pea bean). Susceptible to curly top.

In certain heterozygous families of this cross all grades of resistance to both diseases appear.

Species resistant to curly top. Four species of beans were tested for curly top, including common beans, Butter beans, Small Lima or Baby Lima, and Blackeye cowpea. All species included susceptible varieties, but the common bean appeared to be most susceptible, although immune and highly resistant varieties were found. The Butter bean appeared moderately susceptible in the limited number of varieties under test. The Baby Lima did not show the extremes of susceptibility, but no highly resistant or immune varieties were discovered in the limited number tested. There is evidence for the belief that a wide range of resistance may be found in all species of commercial beans.

A wide range of resistance to curly top was found in the common beans. Among the white beans only 4.7 per cent were found in the highly resistant Grade 1. Among the mottled beans 20 per cent were included in Grade 1, but in the red and pink varieties 35 per cent were found highly resistant. In Grade 5, which includes those beans that were destroyed by curly top, the percentages were as follows: white varieties, 52 per cent; pink and red, 29 per cent; and mottled, 30 per cent.

CONCLUSIONS

1. Curly top of sugar beets attacks varieties of all the commercial species of beans.

TABLE 2.—Summary of resistance to curly top

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Grades of resistance	Immi no v dar	Immune or no visible damage	SI; in;	Slight	Consid	Considerable	Heavy injury	uvy ury	Destr	Destruction	
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	ties		ties	٠.	ties	age	ties		ties	age	
P. vulgaris White	3	4.7	အ	4.7	6	14.0	16	25.0	33	51.6	
Pink or Red	20	35.6	C 7	14.2	0 1	14.2	Н	7.0	4	29.0	
Mottled	¢.1	20.0	က	30.0	H	10.0		10.0	က	30.0	
P. multiflorus White			H	100.0							
P. lunatus sieva White	0	0.0		17.0	4	0.99	Ħ	17.0			

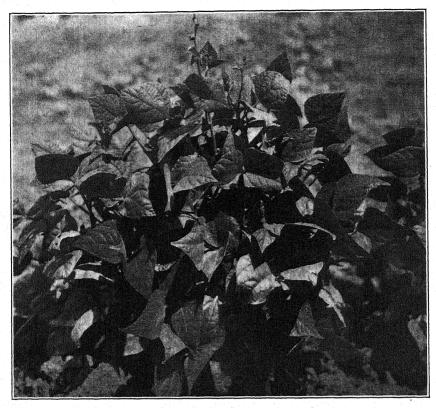


Fig. 6. California Pink. Resistant to curly top.

- 2. Highly resistant varieties were found in the species of common beans and in the small Limas.
- 3. Resistance to curly top is partially but not exclusively correlated with pink and red colors. White beans, highly resistant to curly top, have been found.
- 4. A combination of resistance to both curly top and mosaic is possible in a single variety of beans.

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PHYTOPHTHORA ARECAE, PARASITIC ON ARECA TOPS, AND A STRAIN OF P. PALMIVORA BUTL. (P. FABERI MAUBL.) ON A NEW HOST. ALEURITES FORDI

S. V. VENKATARAYAN

INTRODUCTION

Phytophthora arecae. Towards the end of the year 1929 a certain amount of killing of the tops of the Areca palm, Areca catechu L., were reported from the Marthur Experimental Garden in the Mysore malnad, or the portion adjoining the western ghats. The garden is a small one of about 5 acres comprising 2,000 trees. Investigation showed the killing to be due to a species of Phytophthora, and it seemed desirable to see whether this was the same species as that affecting the nuts, namly, Phytophthora arecae (Colem.) Pethyb. It was with this purpose in mind that the present investigation was taken up.

HISTORICAL

Phytophthora arecae has been known to affect the Areca palm in two ways: the one resulting in the fall of the nuts, and the other in the death of the tops, or bud rot. Coleman (2) describes two methods of infection of the tops, one by means of the stalks of the bunches of fruits and the other by means of leaf sheaths surrounding the growing point. He found the first method to be more usual than the second but showed by inoculation experiments that the second method was a possibility. The attack on the tree tops, however, was rare, and he accounts for this by the possible washing off of the zoospores by the heavy rains of the monsoon. He does not mention the time of year, if any, when the dying of the tops is at its worst.

Butler (1), working on the bud rot of palms in India, records the fact that, according to popular opinion and his own observations, there is a seasonal prevalence of the disease. He says that the number of cases of infection is greater during the period August to February than during March to July and that humidity seems to be a very important factor for infection. Similarly, Gadd (4), working on the bud rot of Areca in Ceylon, mentions the occurrence of the disease in the month of December. Our experience goes to show that there is a seasonal prevalence of the disease on Areca nut, also. In the Marthur Farm approximately 40 trees died between the months of October, 1929, and February, 1930, while previous to this and even later, no cases were reported. Again, it was only in December, 1930, that the first case of infection for the year was reported from the same place. Another interesting observation by Butler (1) is that

the infection may remain latent for 2 years so that unless scrupulous care is taken in destroying all infective material from the garden and its surroundings, the infection may recur after the lapse of some time.

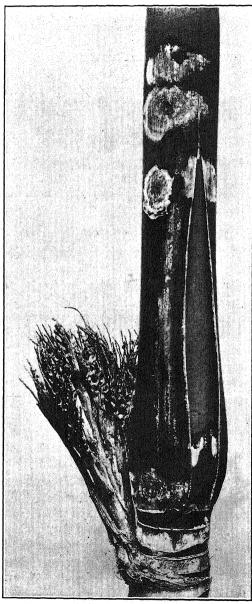


Fig. 1. The top of an Areca palm showing the infection of the leaf sheaths. The Phytophthora growth may be seen both at the base and the top of the outer sheath as well as in the second sheath in between the margins of the outer sheath.

SYMPTOMS

The outer sheaths of infected tops revealed the presence of mycelium and sporangia of the Phytophthora type, and hyphae were found also in the cells of the infected tops. Both the types of infection described by Coleman (2) were found, those in which the tops showed a watery and slightly discolored appearance at the base of the flower stalks and those in which the bases of the outer leaf sheaths were rotten and could be pulled out quite easily. (Fig. 1.)

ISOLATION

Aseptic pieces of the infected leaf sheaths and the portions at the junction of the discolored and apparently healthy tissue in the tree top, planted in poured plates of malt agar, yielded a fairly pure growth of Phytophthora. The fungus grows well on Quaker-oats agar and French-bean agar but not so well on malt agar. Subsequent transfers were all made to Quaker-oats agar.

THE FUNGUS

Sporangia were rather scanty on the solid media but formed within 24 hours when transferred to a Petri dish containing sterile distilled water. They averaged 38.5 x 29.5 µ (average of 80 measurements). The extremes were 26.4×22.2 , 55.5×42.0 , and $62.7 \times 39.0 \,\mu$. It is interesting to compare these measurements with those given by Coleman (2), which are 30.1×20.6 , 51.2 x 45.4, and 71.0 x 43.3 µ. No oospores have yet been found in any of the cultures of this fungus from January, 1930, when culture work was commenced. In December, 1930, and January, 1931, chlamydospores were noticed in a subculture transferred in October, 1930. A subculture made in December of the same year showed the formation of chlamydospores in about 20 days. Transfers made prior to October also showed a few chlamydospores when examined after this date, although, during previous examinations, they were never seen. They were not so numerous as they commonly have been in cultures of Phytophthora Faberi Maubl., and, as far as we now can judge their production seems to be seasonal. The chlamydospores possess thick walls. They have 3 walls (Fig. 2, D), as seen by Dastur (3) for P. parasitica Dastur; the outermost is thin and hyaline, the central thick, and the innermost thicker than the outermost but thinner than the central. The contents are finely granular, with no indication of oil globules. They measured from 24-42 µ, with an average of 34.3 µ (average of 19 measurements).

Oospores in mixed cultures. As oospores were not formed in any of the pure cultures of this fungus it was decided to attempt paired cultures with single-spore strains of the Areca and the sandal Phytophthora isolated by

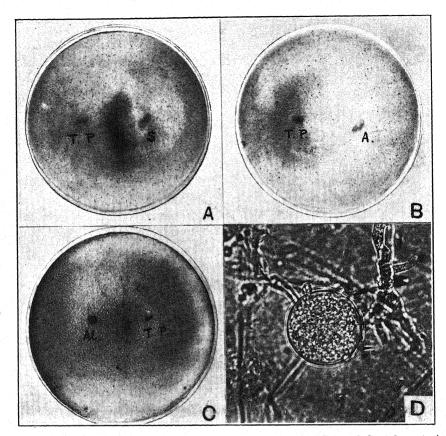


Fig. 2. A. Paired culture of the top-rot Phytophthora (T.P.) and Santalum strain (S). Five-days-old culture on French-bean agar, lighted from underneath. Shows brown line in the middle, which is the area of the greatest development of oospores. B. Paired culture of the top-rot (T.P.) and Areca nut (A) strains. Five-days-old culture on French-bean agar, lighted from underneath. No brown line was formed showing the identity of sex in the 2 strains. C. Paired culture of the top-rot (T.P.) and Aleurites (Al) strains. Seven-days-old culture on French-bean agar, not lighted from underneath. Shows brown line. Compare with figure 4, C. D. Photomicrograph of a chlamydospore of the top-rot Phytophthora. ×600.

Narasimhan (7). In mixed cultures of the fungus from the Areca-tops strain, oospores were formed with the sandal (Fig. 2, A) but none with the Areca strain (Fig. 2, B). Oospore formation was always accompanied by the appearance of the characteristic brown line at the junction of the 2 mycelia, as observed by Narasimhan (7) for his heterothallic strains. The antheridia were all of the amphigynous type. The oogonia made their

¹ I have to thank Mr. M. J. Narasimhan for kindly allowing me to use his isolations of Phytophthora from Areca and sandal in the paired cultures.

1932]

TABLE 1.—Measurements of oogonia and oospores of Phytophthora arecae

	Oogonia	Oospores	Number of measurements
Top-rot Phytophthora plus P. on	(diameter in µ)	(diameter in μ)	
sandal	28.9	25.5	40
P. arecae plus P. on sandal	32.6	28.1	25
P. arecae plus P. on sandal (Nara-			
simhan) (7)		30-31	
P. arecae (Coleman) (2)		23-36	
P. arecae (Rosenbaum) (8)		29.45-31.49	

DISCUSSION

Gadd (4), working on Phytophthora arecae causing bud rot of Areca palms in Ceylon, did not obtain oospores either in pure culture or in mixed cultures with any of the 7 strains of P. Faberi and with P. palmivora from coconut. He found in old cultures spherical bodies resembling chlamydospores produced less abundantly than in P. Faberi. In this respect the budrot Phytophthora he had under observation seems to agree with that found in Mysore. The diameter of these bodies he gives as 24-48 u. Chlamydospores have not been noticed in P. arecae either by Coleman (2) or Rosenbaum (8). This absence of chlamydospores in P. arecae, together with the fact that both Coleman and Rosenbaum found oospores in this fungus, led Gadd to presume that the bud-rot fungus in Ceylon might not be P. arecae but P. Faberi, P. palmivora, or a new allied species, unless chlamydospores should be found in P. arecae under certain circumstances. Narasimhan (7) has suggested that P. arecae might be heterothallic and that the male and female strains might possibly tend to get isolated on different hosts by the loss of one or other of the sex strains. If such be the case, it is possible that in Ceylon, where P. arecae is a much later introduction than in Mysore, there is only one of the sexes, and, to compensate for the loss of sex, the fungus assumed the ability to produce vegetative resting spores in the form of chlamydospores. Now that chlamydospores have been noticed in cultures of P. arecae producing bud rot in Mysore and that this fungus behaves in respect to heterothallism in the same manner as the strain on the Areca nut, from which P. arecae was originally described, Gadd's supposition about the identity of the bud-rot fungus seems to me to be no longer warranted. A more important reason is that Coleman (2) gives as the habitat of P. arecae the bases of leaves, fruits, peduncles, and apex of Areca catechu, while Gadd (4), himself, says that the fungus from the bud tissues and that from the nuts are indistinguishable in culture.

PHYTOPHTHORA ON ALEURITES FORDI

INTRODUCTION

Towards the end of October, 1930, seedlings of Aleurites fordi Hemsl.² (the tung-oil tree of commerce) were found to bear leaf spots (Fig. 3). Infected leaves wilted gradually and finally dropped down, leaving the seedling bare of all leaves in extreme cases. In the early stages the leaves show water-soaked areas, either near the margin or the tip, and the spots

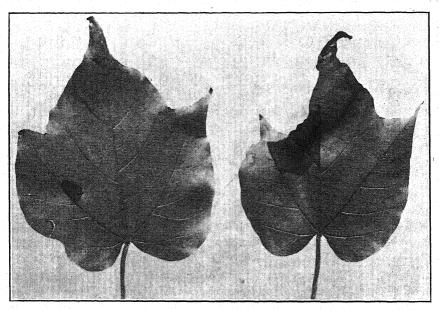


Fig. 3. Two leaves of *Aleurites fordi* Hemsl. showing natural infection due to Phytophthora.

² These seedlings were raised by Mr. M. G. Venkata Rao, Assistant Conservator of Forests on sandal-spike duty, from seeds that were obtained from Kew. They were about 4 months old at the time of infection and were growing in pots in the compound of the agricultural laboratories at Bangalore.

later become brown. They are at first small, limited by the prominent veins, and gradually spread and involve almost the whole leaf. Phytophthora mycelium and sporangia were noticed on the under side of affected leaves. This Phytophthora on A. fordi is interesting in that this is the first time, as far as can be made out from the literature, that it has been noticed on this host. There are other members of the family Euphorbiaceae, to which Aleurites belongs, that are attacked by Phytophthora, such as Ricinus communis L. (3), Hevea brasiliensis Muell. (5, 6), Manihot Glaziovii Muell., capable of artificial infection only by P. Meadii McRae (5) and Jatropha curcas L. (7, 9).

ISOLATION

Affected leaves in the early stages of attack were selected and washed well under the tap. Pieces from two of these leaves involving the apparently healthy and the discolored portions were washed in sterile distilled water and transferred to Petri dishes containing malt agar. The resulting growth was pure, and transfers were made to Quaker-oats agar in tubes.

THE FUNGUS

The mycelium grew very well on common media, such as Quaker-oats agar and French-bean agar, there being plenty of aerial mycelium. On potato-dextrose agar and malt agar the fungus did not grow so well as on the above-named media. The growth on carrot agar differed slightly from that on others and from the growth of the top-rot Phytophthora on this same agar (Fig. 4, C). The Aleurites strain showed concentric rings on the carrot agar, with a little spreading growth around the inoculum, whereas the top-rot Phytophthora showed only a spreading growth. In the other media the Aleurites strain showed only a spreading growth. developed on Quaker-oats agar but more readily in sterile distilled water in Petri dishes. They measured on the average 32.0 x 23.6 u (average of 35 measurements), with extremes of 54.6 x 32.7 and 20 x 15 u. Chlamydospores were formed in large numbers on Quaker-oats agar. They were both terminal and intercalary. They contained a lot of oil globules unlike those of P. arecae from the tops (Fig. 4, D). Oil globules have been noticed by Dastur (3) in the chlamydospores of P. parasitica. The chlamydospores have 3 walls, the outer very thin and transparent, the central thick and vellowish, and the inner one, which is thinner than the middle one but thicker than the outermost. Dastur (3) says that the outer wall can be seen only when stained and that the innermost wall is not closely attached to the central, thick wall. In the cases observed by me the innermost wall was found closely attached to the central wall, and there was no necessity to stain the chlamydospores to see the outermost hyaline wall, as may be seen from the photograph, which is of an unstained preparation (Fig. 4, D). They measured on the average 30.9 μ (average of 49 measurements) ranging between 15 μ and 43.8 μ .

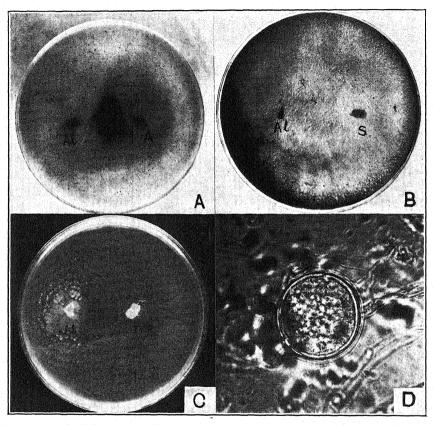


Fig. 4. A. Paired culture of the Aleurites Phytophthora (Al) and Areca nut (A) strain. Five-days-old culture on French-bean agar, lighted from underneath. Shows brown line of oospore region. B. Paired culture of the Aleurites Phytophthora (Al) and Santalum (S) strains. Nine-days-old culture on French-bean agar, not lighted from underneath. No line seen showing the identity of sex in the 2 strains. C. Paired culture of the Aleurites Phytophthora (Al) and top-rot Phytophthora (T.P.). Nine-days-old culture on carrot agar. Oospores developed in 7 days without the accompaniment of the brown line. Note the difference in the mycelial growth of the 2 strains on this agar and compare with figure 2, C. D. Photomicrograph of a chlamydospore of the Aleurites Phytophthora. ×600.

Oospores. Oospores have not been found in cultures nor in the affected leaves thus far examined, but they develop readily in 3 days in mixed cultures on French-bean agar with *Phytophthora arecae* (Fig. 4, A) and

the Phytophthora of the top rot of Areca (Fig. 2, C). In all these cases the characteristic brown line was formed. No oospores were formed in mixed cultures with the Phytophthora on sandal leaves (Fig. 4, B). The antheridia were all of the amphigynous type. The following table shows the measurements of the oospores and their relation to the oospores formed in the heterothallic *P. arecae*.

TABLE 2.—Average diameters of oogonia and oospores in mixed cultures of the Aleurites

Phytophthora and P. arecae

	Oogonia	Oospores	Number of measurements
Aleurites Phytophthora plus P. arecae Aleurites P. plus top-rot strain Top-rot P. plus sandal strain (writer) P. arecae plus sandal strain (writer) P. arecae plus sandal strain (Narasimhan) (7) P. arecae plus Jatropha strain (Narasimhan) (7)	(diameter in μ) 27.8 25.8 28.9 32.6	(diameter in μ) 23.04 22.2 25.5 28.1 30-31 26-27	25 40 40 25

It will be seen that the oospores in mixed cultures of the Aleurites Phytophthora and P. arecae are smaller than those formed in mixed cultures of the Areca and sandal strains of P. arecae, and more nearly approach in size those formed between the Areca and Jatropha strains of P. arecae as given by Narasimhan (7).

INFECTION

A preliminary infection experiment was carried out with Aleurites fordi seedlings in a moist chamber in the laboratory. One leaf was inoculated on the under surface and another on the upper with 2 loopfuls of zoospore suspension. The check was inoculated in the same manner with sterile distilled water. On the third day the leaf inoculated on the under surface showed dark patches around the inoculated point, while that inoculated on the upper surface and the check remained healthy during the course of the experiment, which lasted 7 days. The fungus was reisolated from the inoculated leaf. It showed the same characteristics as the original isolation. Chlamydospores were first formed in 10 days, increasing in abundance in 17 days to 3 weeks.

RELATIONSHIPS

The abundance of chlamydospore formation in the cultures of the Aleurites Phytophthora leads me to think that it is Phytophthora palmivora Butl. (P. Faberi Maubl.). Since it forms oospores readily with the Areca strain of P. arecae, which has been definitely shown to be the male of a heterothallic species by Narasimhan (7), we may presume that the Aleurites strain is a female, probably of a different species. The oospores look like hybrids, as may be judged by their size (Table 2). The condition resembles that seen when cultures of P. parasitica, which have lost their capacity to form oospores individually, are mated with P. arecae, when oospores intermediate in size between those of P. parasitica and P. arecae are formed, as mentioned by Narasimhan (7). In this connection we may compare the mycelial growth of P. arecae and the Aleurites strain in French-bean agar and carrot agar (Figs. 2, C, and 4, C). So this fungus on Aleurites probably is not P. arecae but a strain of P. palmivora Butl. (P. Faberi Maubl.).

I am greatly indebted to Dr. Leslie C. Coleman, M.A., Ph.D., Director of Agriculture, Mysore, and Mr. M. J. Narasimhan, Officiating Mycologist, for several helpful criticisms and suggestions during the course of this work.

SUMMARY

Phytophthora arecae was isolated from dying Areca tops. The fungus formed oospores in mixed cultures with the heterothallic strain on Santalum album but not with that on the Areca nuts, hence proving its identity with the latter strain.

Chlamydospores have been discovered in the cultures of the fungus of the Areca tops in Mysore, as has already been done for the bud-rot Phytophthora in Ceylon.

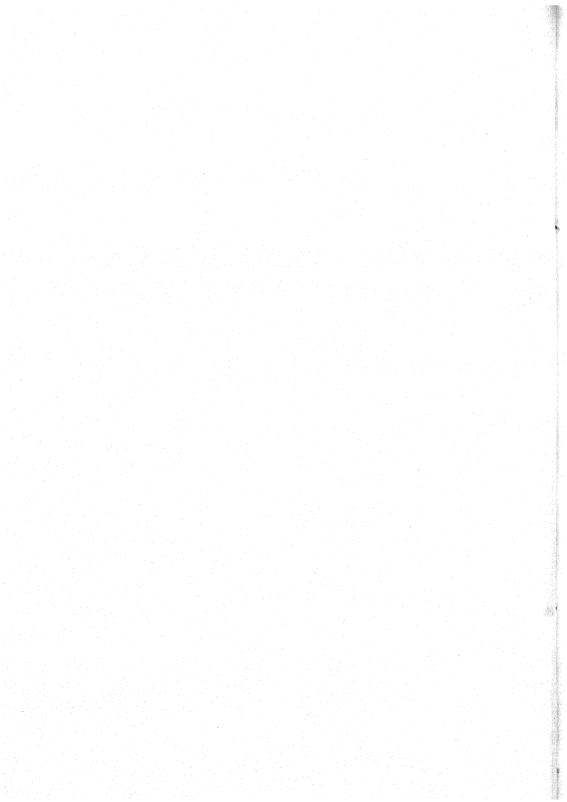
A strain of *Phytophthora palmivora* (*P. Faberi*) has been noticed on a hitherto undescribed host, namely, *Aleurites fordi*, the tung-oil tree. In mixed cultures it seems to hybridize with the male strain of *P. arecae*.

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AREOLATE MILDEW OF COTTON

JOHN EHRLICH' AND FREDERICK A. WOLF

In 1890 Atkinson (1) briefly described a disease of cotton found by him in Alabama, the causal organism of which he designated *Ramularia areola* Atk. All subsequent reports of this disease by various investigators have dealt primarily with its occurrence and the probable losses caused by it, but nothing has been contributed toward a better understanding of the morphology and development of the pathogen. Accordingly, it was deemed advisable to make such an investigation, and the present paper is the result of these studies.

NAMES

This disease has been given various names, including "areolate mildew" by Atkinson (1), "frosty blight" and "areolate mildew" by Duggar (6, page 296), "mildew" by Cook (5), "false mildew" by Vincens (21), and "gray mildew" by Laycock (14). Of these, areolate mildew appears to be preferable and, accordingly, is used in this report.

SUSCEPTS

Since no effort has been made by any one to determine the suscept range, there are no data on this point. The pathogen is not known to occur, however, except on species of Gossypium.

The organism was first reported on the leaves of cultivated upland cotton, Gossypium hirsutum L. It is not confined to this species, however, since Farquharson (7) has observed that American cotton in Southern Nigeria is very commonly attacked, while it does not seriously affect such native cottons as G. peruvianum Cav. and G. vitifolium Lam. Vincens (21) reported that the fungus occurred on imported varieties of cotton in the Botanical Gardens of Saigon in Indo-China but left the indigenous varieties practically unharmed. Sea-island cotton, G. barbadense L. also is known to be a suscept.

IMPORTANCE AND RANGE

Areolate mildew is a disease of the enphytotic type, which may be found every season practically wherever cotton is cultivated. In spite of this, the actual losses from it are slight or inappreciable, except on rare occasions and in limited localities. Usually it involves only that part of the crop that is situated in low-lying portions of the field.

Since the discovery of areolate mildew near Auburn, Alabama, it has

¹ This investigation was conducted while the senior writer held a graduate fellowship at Duke University, Durham, N. C.

been collected, according to the records of the Division of Mycology and Disease Survey of the Bureau of Plant Industry, United States Department of Agriculture, in the following Cotton Belt States: Louisiana, Mississippi, Florida, Georgia, South Carolina, North Carolina, Tennessee, and Arkansas. There are, in addition, various reports from the West Indies.

The first collection in South America was made by Spegazzini² in Paraguay and Brazil in 1892. He described the pathogen as *Cercosporella Gossypii* Speg. A report from São Paulo, Brazil (15), states that cotton leaves there are attacked by *Ramularia aérea*, presumably, an incorrect spelling of *R. areola* Atk.

Birmingham and Hamilton (4), in 1923, noted the occurrence of areolate mildew in Australia. The disease has been reported for a number of years from various parts of Africa. Farquharson (7), in 1914, noted that it was very commonly present on American cotton in Southern Nigeria, as previously stated. Two years later, Small (16) recorded its occurrence in Uganda, chiefly on young plants. His later reports (17, 18) state that mature leaves of cotton are severely attacked, especially during prolonged wet periods. Others who have recorded its occurrence here include Snowden (19), Laycock (14) and Hansford (8), the latter of whom stated that it was very commonly present but that it caused little damage.

Wallace (22) observed areolate mildew in Tanganyika and Swainson-Hall (20) in West Africa, and various collections have been made in the Belgian Congo.

In Asia the records of Vincens (21) show its occurrence in Cambodia and in the Botanical Garden of Saigon.

SYMPTOMS

Areolate mildew usually appears on the leaves toward the close of the growing season and may be distinguished readily from all other cotton diseases. Its lesions are irregular, angular areas bordered by the veinlets and are light green to yellowish green when viewed from the upper surface. They have the appearance of being covered by mildew when seen from the lower surface, due to the profusion of conidiophores and conidia (Fig. 1). Occasionally the white coating appears also on the upper side. Lesions of similar appearance may form on the bracts surrounding the bolls.

The defoliation, which may follow from abundant lesions on the leaves, may result in the premature opening of the bolls and the consequent deterioration of the staple, as has been suggested by several observers.

THE PATHOGEN

The present studies show that the fungus that causes areolate mildew possesses three stages in its cycle of development, a conidial stage, described

² Saccardo, P. A. Sylloge Fungorum 10: 565. 1892.

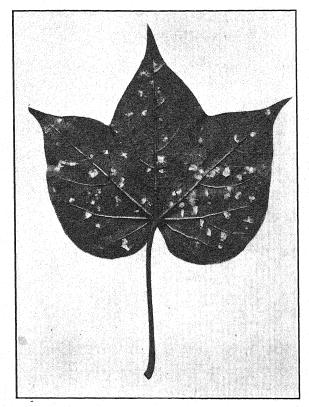


Fig. 1. Areolate mildew of cotton. (Courtesy of W. W. Gilbert).

by Atkinson (1), in 1890, as Ramularia areola and as Cercosporella Gossypii by Spegazzini in 1892, which appears on the living tissue; a spermogonial stage, which occurs in autumn on the fallen leaves; and an ascigerous stage, which develops in the spring on decaying leaves.

Conidial stage. Microscopic examination shows that clusters of hyaline conidiophores, 25 to 75 \times 4.5 to 7.0 μ , occur on the lower surface of lesions, rarely on both surfaces. They bear 1- to 3-septate, hyaline conidia, 14 to 30×4 to 5 μ (Fig. 2, E, F), either terminally or laterally, which may remain in chains or occur singly.

Paraffin sections show that the conidiophores emerge through the stomata and arise from a loosely compacted, substomatal stroma. The fascicle is so constricted at the stomatal orifice as to look like an hourglass (Fig. 2, A). Hyphae ramify from the stroma, coursing both between and within the spongy and palisade parenchyma, and may so closely intertwine with those radiating from neighboring centers as to form an almost continuous stroma within the limits of a single lesion.

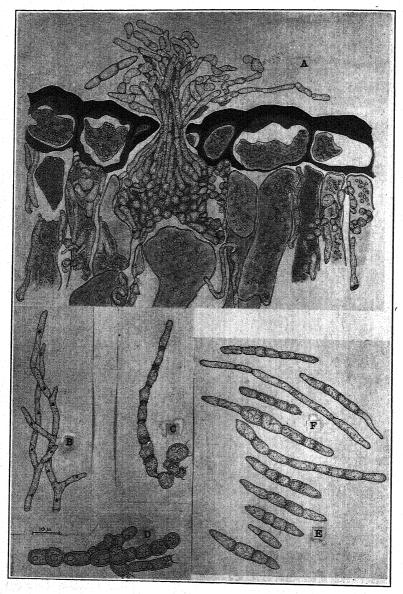


Fig. 2. A. Upper surface, in vertical section, of cotton leaf affected with areolate mildew. The conidiophore fascicle arises from a substomatal stroma and extends to the surface through the stomatal orifice; mycelium both between and within the palisade parenchyma. B. Aerial mycelium from culture. C. Hyphae with plate-like septa at the surface of the agar. D. Hyphae from beneath the surface of the agar. E. Conidia. F. Germinating conidia.

A destruction of invaded cells accompanies the advance of the mycelium. In newly attacked cells the protoplasm becomes more granular and is later plasmolyzed. The middle lamella is manifestly dissolved, as shown by the separation of suscept cells. The cells finally collapse completely and disintegrate, leaving only fragments of the secondary membranes.

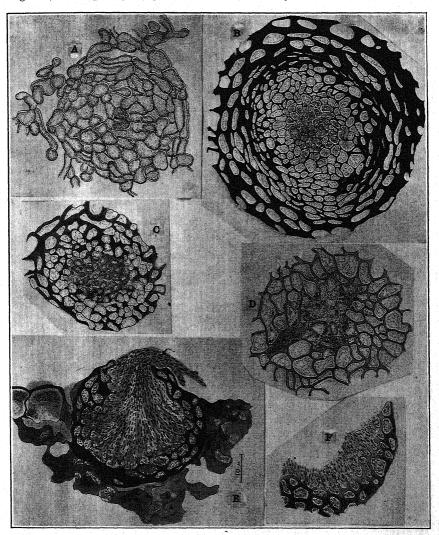


Fig. 3. A. Median section of young sclerotium in culture, an early stage in formation of spermogonium. B. The walls of the cortical cells of the developing spermogonium have become thick and those of the medullary portion densely filled with protoplasm. C and D. Early stages in formation and liberation of spermatia. E. Mature spermogonium in vertical section with spermatia embedded in a mucilaginous matrix. F. Section of the spermatiferous area of the inner wall of a spermogonium.

Collections of leaves bearing an abundance of conidia were made in October, 1930, and placed in wire baskets out of doors. In April, 1931, a whitish coating of conidia was present on same of these. When these conidia were placed in water, they germinated, thus showing that viable conidia are at hand in the following growing season.

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Spermogonial stage. By the time the leaves have withered and fallen and becomes brown, the lesions appear as sharply defined, darker areas. An examination of the lower surface with a hand lens reveals numerous slightly raised black spots, the spermogonia. They are found to be mature throughout November and December. They measure 28 to 75 μ in diameter, and, when crushed on a slide under a cover-glass, numerous spermatia, 2.4 to 4.0×0.4 to $2.0~\mu$, ooze out. Attempts to induce germination of spermatia met with consistent failure.

Sections of affected tissues during this period show various stages in the development of spermogonia. At an early stage they are sclerotium-like bodies composed of compact thick-wall cells (Fig. 3, A). The walls of the innermost cells, which are the first to become spermatiferous, next become thinner and their cell content more densely granular and deep-staining (Fig. 3, B). Each protoplast eventually becomes separated into a number of rod-shape or bone-shape spermatia, after which the cell wall disintegrates and the spermatia are liberated in a matrix consisting of cytoplasm and disintegrating cell walls (Fig. 3, C, D, F). This form of spermatia and their liberation proceed centrifugally until only the outer layers of thick-wall cells remain, forming the spermogonial wall. With the apical opening of the spermogonium the spermatia are exuded in a viscous mass through the ostiolum (Fig. 3, E).

This endogenous formation of spermatia (variously designated pycnospores, microconidia, and stylospores) has been previously described, apparently, only by Laubert (13) for an unnamed organism occurring on dying apple branches and by Klebahn (12) for Mycosphaerella Hieracii (Sacc. et Br.) Jaap. Laubert states: "die Sporen dadurch entstehen, dass sich die Zellinhalte des anfangs vorhandenen, farblosen Paraplechtenchyms zu dünngleichzeitig die Membranen der Mutterzellen verquellen und vergallerten." Klebahn describes the origin of "Mikroconidien," "als ob sie durch Zerfall der Hyphen eines feinfädigen Geflechts, das das Innere der Sklerotien, vermischt mit weiteren Zellen, auffüllte, entstanden waren."

Ascigerous stage. The perithecia are sparingly present in April on the lower leaf surface in the areas previously occupied by the conidia and spermogonia. They have been noted in each of the three seasons in which this organism has been studied. They appear to arise from stromatic or sclerotial structures embedded within the tissues of the leaf. These

sclerotia occur in abundance throughout the fall, winter, and spring. They are first noted among the spermogonia, and no means has been determined of knowing which of these are destined to become spermogonia or perithecia or which are to remain as sclerotia.

Mature perithecia are dark brown, with a slight papilla, and measure 70 to $80 \,\mu$ in diameter. They project well from the leaf surface (Fig. 4, A).

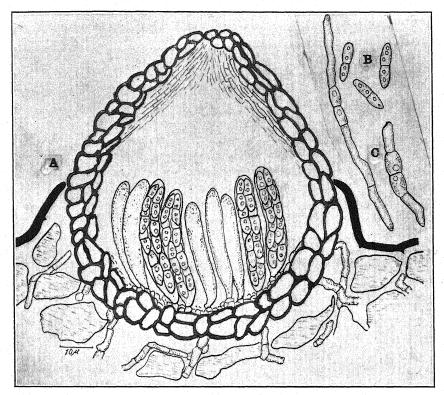


Fig. 4. A. Median section of a perithecium of Mycosphaerella areola. B. Ascospores. C. Germinating ascospores.

The asci are 35 to 40×6 to 8 μ , and the biseriate ascospores are elongated and slightly constricted at the septum, which divides the spore unequally (Fig. 4, B). They measure 12.4 to 15.6 \times 3.2 to 3.8 μ .

Growth in culture. Attempts to isolate the pathogen in pure culture by the use of conidia led to the conclusion that it is a difficult procedure. In the greater proportion of trials, the conidia failed to germinate when placed in tap water or distilled water, even when bits of cotton leaves or traces of sugar were added. In some cases a small proportion had begun to grow after 8 to 10 hours. When such germinating conidia (Fig. 2, F) were

spread over the surface of cotton-leaf-decoction agar, slow-growing, grayish white, hemispherical colonies, 1 to 3 mm. in diameter, appeared after several weeks. The aerial mycelium was slightly fluffy and consisted of slender, thin-wall hyphae of uninucleate cells (Fig. 2, B). At the level where the aerial hyphae enter the substratum, the walls thicken, and the septa appear as plates, which extend beyond the lateral walls (Fig. 2, C). Within the substratum the mycelium is very compact and is made up of rounded thick-wall cells filled with oil globules (Fig. 2, D). In a series of cultures planted on October 23, 1928, both conidia and spermogonia bearing spermatia had formed by December 15. These conidia were like those present on leaf lesions. Paraffin sections of such colonies on cotton-leaf-decoction agar show that spermogonial development in agar is of the same type as occurs on leaves.

Isolations from ascospores were made by inverting agar plates over decaying leaf fragments, placed on moist blotting paper in the tops of Petri dishes. The ascospores, which were forcibly ejected and were lodged on the surface of the agar, where they had begun to germinate (Fig. 4, C) after 15 hours, were transferred to potato-agar slants. Here they developed into colonies of the same type of growth as those isolated from conidia. They have, however, not been observed to form either conidia or spermogonia.

Pathogenicity. All attempts by means of artificial inoculations to establish the pathogenicity of the areolate-mildew organism have been unsuccessful. Suspensions of conidia, both from leaves and from culture, have been placed by means of a camel's-hair brush on both the lower and upper surface of young and mature, healthy cotton leaves. The inoculated plants were then covered with bell jars or wet cotton was placed on the leaves, after which they were wrapped in wax paper.

Blocks of agar in which ascospores had been discharged were also placed on leaves and protected against too rapid desiccation in the same manner. Failure to secure infection is doubtless due primarily to lack of suitable moisture conditions, as indicated by all observations to the effect that wet seasons are necessary for the occurrence of the disease in the field.

DISCUSSION

Of the approximately 400 species of Ramularia that have been described, the perithecial stage is known for less than a dozen. Killian (10) states that Ramularia is characterized, in general, by the complete suppression of the ascigerous stage and that those species in which it persists produce perithecia rarely or under special conditions, only. Those whose perithecial stage is known belong with one exception to the genus Mycosphaerella. This exception is Fabraea Ranunculacearum Fr., which appears in autumn

on Ranunculus repens E. & E. Killian (11) showed that its conidial stage is Ranularia repentis Oud., in confirmation of the earlier observations of Goussewa, and he noted, in addition, the presence of a pycnidial (spermogonial) stage.

In his earlier studies dealing specifically with Ramularia Geranii (West) Fekl., R. Adoxae Rabenh., R. Saxifragae Syd., R. variabilis Fekl., R. Lapsanae Desm., and R. Parietariae Passer., Killian (9) concluded that most Ramularias produce a second type of reproductive organ, the sclerotium. He regards sclerotia in various stages of development as a transition leading toward perithecia. Some species, as R. variabilis, normally produce conidia, spermogonia, sclerotia, and perithecia, and might be properly regarded as complete-cycle forms; others, as R. Saxifragae, produce only conidia and sclerotia; still others, as R. Parietariae and R. Geranii, form conidia, only. The latter of these is known to produce conidia or conidiophores on decaying leaves in the spring. It may be recalled at this point that viable conidia of the cotton Ramularia are present in the spring on conidiophore fascicles. It, therefore, would appear from the studies made on Ramularia that relatively few species bear perithecia and that certain of these, as the fungus here under consideration, produce them only sparingly.

Two other well-known species, Mycosphaerella Fragariae (Tul.) Lindau (Ramularia Tulasnei Sacc.) and M. Hieracii (Sacc. et Br.) Jaap. (R. Hieracii (Baumler) Jaap.) have been noted to produce ascospores within the perithecia and conidia at their surface.

While adequate proof of the connection of the conidial stage of the cotton fungus with the perithecial stage is lacking, the evidence in hand warrants one in regarding them, with a good degree of certainty, as stages of the same organism. This evidence may be summarized as follows: (1) Isolations from conidia and from ascospores produce colonies of similar appearance; (2) when infected leaves are allowed to overwinter out of doors an ascogenous stage appears in the spring in the areas previously occupied by the conidial stage and then the spermogonial stage, this having been noted during each of the three seasons during which this study has been in progress: (3) with the exception of Ramularia repentis, all species of Ramularia whose ascigerous stage is known have been found to belong to Mycosphaerella.

The perfect stage of the areolate-mildew fungus has apparently not been described previously. Moreover, it is highly improbable that the organism under consideration occurs on species outside the genus Gossypium, since Ramularias are all known to be restricted in their suscept range. The only

Mycosphaerella known to occur on cotton is M. Gossypina Atk. (3), type specimens of which were examined.³

These specimens, collected in autumn, show both Cercospora and mature Mycosphaerella in abundance in the same lesion. Further, the ascospores of *M. Gossypina* are somewhat larger than and of a different shape from those of the areolate-mildew fungus. For these reasons the name *Mycosphaerella areola* is proposed for the areolate-mildew organism with the following brief description:

Mycosphaerella areola, n. sp.

Syn. Ramularia areola Atk.

Cercosporella Gossypii Speg.

Status asciger:

Perithecis hypophyllis, paucis, in maculis aggregatis, parenchymate foliorum immersis, epidermide primo tectis dein suberumpentibus 70–80 μ diam.; aseis fasciculatis, oblongis, 35–40 \times 6–8 μ , 8 sporis; sporidiis distiehis, oblongis, utrinque obtusciuscle rotundatis, inaequaliter didymis, loculis guttulatus, non vel vix subconstrictis, hyalinis, 12.4–15.6 \times 3.2–3.8 μ , Hab. in foliis putridis Gossypii sp.

Status spermatiferus:

Spermogonia dense gregaria in foliis siccis, maculas definite occupans; 28–75 μ diam.; spermatiis hyalinis, 2.4–4.0 \times 0.4–2.0 μ

Status conidicus:

Plagulis superiore primo pallidis inferiore effuso-candidis, angulosis, irregularibus, venulis foliis limitatis, 3–4 mm. diam., saepe confluentibus; hyphis hypophyllis, raro amphiginiis in caespitulos laxe aggregatis, erectis, saepe basi ramosis v. rareus supra denticulatis, pluries septatis, hyalinis, $25–75\times4–7~\mu$; conidiis cylindraceo-ellipticis, utrinque saepe abrupte accuminatis, 1–3-septatis, primitus concatentatis, hyalinis, 14–30 × 4–5 μ . Hab. in vivis foliis Gossypii sps.

SUMMARY

Areolate mildew is a common cotton disease of minor importance that occurs on various species of Gossypium, including G. hirsutum, G. barbadense, G. peruvianum, and G. vitifolium. It has been observed throughout the cotton-growing areas of the southeastern United States, the West Indies, Africa, Australia, and Indo-China.

Areolate mildew is characterized by the presence of angular, whitish patches, usually on the lower leaf surface, which, as a rule, are first evident toward the close of the growing season.

³ Examination of type specimens was made possible through the courtesy of Dr. H. M. Fitzpatrick.

The pathogen, known in its conidial stage as Ramularia areola, occurs on living leaves in late summer; produces the spermogonial stage on fallen leaves during late autumn; and the ascogenous stage appears in the spring, on decaying leaves. Evidence of the connection of the conidial and the perfect stages is presented.

The sclerotia, from which both spermogonia and perithecia arise, are similar in appearance. The spermatia arise endogenously. The perfect stage is herein described as Mycosphaerella areola, n. sp.

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SCLEROSPORA GRAMINICOLA ON BAJRA (PENNISETUM TYPHOIDEUM)

H. CHAUDHURI

Sclerospora graminicola (Sacc.) Schroet. produces "green-ear disease" of bajra in India. As the name signifies, it is characterized by the reversion of whole or part of the ear into a green, leafy condition. Figure 1 shows a photograph of typical green ears of bajra. This disease is very common in the Punjab and often causes serious damage, as it results in complete loss of grain in the affected plants. It was first described in India by Butler (1). He states that it is usually not of sufficient intensity to attract much notice, though at times, particularly in low-lying, ill-drained lands, it causes considerable damage. Mitter and Tandon (12) have confirmed Butler's observations. The writer, in a paper on green ear of bajra (3), showed that the disease was of considerable importance and that the incidence of the epidemic could not be attributed to either bad drainage or special atmospheric conditions. It occurs frequently in all parts of the Punjab. In recent years it has been observed in many more fields in and near Lahore than in 1926–27.

The first symptom of the disease is shown by young plants long before flowering. They show whitening of leaves in long streaks and, when old, the tissues turn brown, tear along the streaks, and, like the disease in Jowar (Andropogon Sorghum Bret.), numerous oospores immersed in the leaf are formed. Badly affected plants do not produce any ear but tufts of leaves instead. Sometimes part of the ear is transformed into twisted narrow leaves (Fig. 1, A), or the whole inflorescence may be shortened and bear only long twisted leaves. The conidial stage of the fungus has not thus far been encountered.

In the Punjab one crop of bajra is raised ordinarily, which is sown in June—July and harvested in October. But sometimes, for fodder purposes, an early crop also may be raised. In September, 1929, the writer found near Lahore a plot of bajra in which all the plants were very badly affected. In that plot not one mature ear was formed. As the plants had not formed any grains, the crop was not harvested at all but was left to stand in the field. On visiting the plot again in early November, the writer found many of the plants dead and, though all of them appeared to be dry and dead, some had developed fresh, leafy, green ears, which came out of the axils of the old ones, now completely dry, and stood out prominently. The development of the green ears twice over in the same plants was no doubt very unusual.

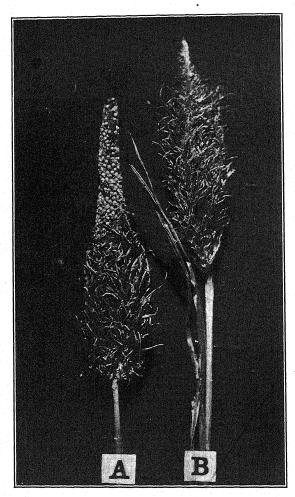


Fig. 1. Two typical green ears of bajra. A. Only half the ear is transformed. B. A completely transformed ear. $\times \frac{1}{3}$.

Diseased plants were brought into the laboratory for study. Sections of every part were cut and examined microscopically. The twisted leaves in the ears did not show any oospores or mycelium, but the ordinary leaves below the inflorescence, which showed white streaks and later shredding, were full of oospores. Figure 2 shows a photomicrograph of a bit of such a leaf. It is full of oospores. The oospores on an average measure $45\,\mu$ in diameter. Inoculation experiments with oospores were performed. Twenty young plants of bajra growing in pots were obtained and 15 of these were inoculated with oospores from bajra of the previous season. Five plants were kept as controls. For inoculation a bit of the shredded

leaf was taken and dipped in mercuric chloride solution (1 in 500) for 5 minutes. It was then thoroughly washed in several changes of distilled water, after which the bit of the leaf was tested in a tube containing sterilized distilled water. Loopfuls of spore suspensions were placed on both surfaces of the experimental plants and in a few cases the leaves were also pricked before the spore suspensions were placed. For 24 hours the inoculated plants, as well as the controls, were kept in a chamber of saturated atmosphere. Then all the plants were removed to the glass house in the laboratory. Some of the inoculated plants showed whitening and later shredding in about 3 to 4 weeks' time. No green ears, however, were

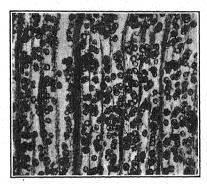


Fig. 2. Photomicrograph of a bit of shredded leaf of bajra, showing numerous cospores. ×35.

formed and the control plants continued perfectly healthy. The shredded leaves of the inoculated plants when examined microscopically showed plenty of oospores.

GERMINATION OF OOSPORES

As Butler (1, 2) mentioned germination of oospores from bajra was unknown and its life history in India was not completely known, the writer tried various methods for germinating the oospores. While at the Sorbonne in 1927–28, he tried to germinate oospores collected during the previous season in India. The oospores after surface disinfection were placed on slides containing ample quantities of various liquids, viz, water, soil decoction, and various synthetic media. These were placed inside sterilized moist Petri dishes and incubated at different temperatures. Only in isolated cases rupturing of oospore walls and formation of abortive germ tubes took place. But no regular germ tubes developed.

During the last three years Hiura (5, 6, 7) and later, Evans and Harrar (4), following Hiura's method, have succeeded in germinating Sclerospora oospores. The writer has also succeeded in germinating the oospores from

bajra in the same way. This was to place a small piece of moist filter paper bearing minute quantities of oospore powder on the surface of a large bit of moistened cotton in the bottom of a Petri dish (care being taken that the contact between the paper and the cotton be only partial) and a second similar layer in the lid of the dish; the space between the two being about half the height of the dish. Figure 3 shows germination of the oospores

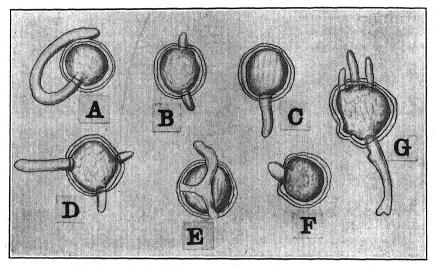


Fig. 3. Germination of cospores. × about 400. A-D. Oospores from 1930 plants. E. From 1929 plant. F. From 1928 plant. G. From 1927 plant.

from bajra. Though Evans and Harrar have sketched germinating oospores with very long germ tubes similar to those described by Hiura, the germ tubes from oospores of bajra Sclerospora were never long. It will be seen that in many cases more than one germ tube developed and figure 3, G, shows an oospore with 4 germ tubes. In germination a portion of the outer coat may be ruptured and the inner coat protrude out as a germ tube or the outer coat may split into several parts before the germ tubes are formed.

VIABILITY OF THE OOSPORES

As the writer had collected and preserved infected bajra leaves every year during the last 5 years, he had the opportunity of studying the period of viability of these bajra oospores. It is interesting to note that even 5-year old oospores did not lose their power of germination. Previously Melhus and others (10, 11) had found Sclerospora oospores viable for 30 months under laboratory conditions. Considering the very thick coat of the oospores, the long period of viability is not surprising.

DISCUSSION AND CONCLUSIONS

Formation of conidial stage in Sclerospora has been reported by many workers. Though the writer has failed to encounter sporangial stages, they have been reported by Butler in India. He found them evanescent and sometimes absent altogether. Kulkarni (9) found the sporangial stages in great abundance on bajra and jowar in Bombay Presidency. The infection was noticeable from the beginning, even when the seedling had put forth only 2 or 3 leaves and conidia were produced only at night. Melhus, Van Haltern, and Bliss (11) observed conidial formation both during night and day whenever certain conditions were fulfilled, viz., (a) complete saturated atmosphere; (b) turgid host tissues; (c) slight moisture film on the surface of the leaves; (d) temperature between 8° and 27° C. Perhaps the failure of the conidial stage of the fungus in the Punjab may be attributed to the absence of a complete saturated atmosphere and also to the absence of a slight moisture film on the surface of the leaves due to very low humidity of the atmosphere in this part of the Punjab.

Regarding infection, Hiura (8) has shown that the disease is of a systemic character, and by inoculation experiments he proved that seedlings may be infected through the roots, coleoptile, and rhizomes. Melhus, Van Haltern, and Bliss (11) found that when oospores overwintered naturally in the field they produced twice as much infection as oospores kept in the laboratory. They showed soil infection took place in almost all cases when plants were grown in untreated soil, but repeated trials on sterile soil gave no evidence to support the possibility of the fungus overwintering in the mycelial stage within the seeds of the host plant. Kulkarni (9) tried inoculation with zoospores in a large number of plants without success. Even when a healthy plant touched a diseased plant in the field, it never got infected. Butler also failed to inoculate healthy leaves with zoospores. These show definitely that in bajra sporangial stages, even though present, are incapable of bringing about any infection, and, as there is no evidence in support of the view that the fungus passed the unfavorable period within the seeds of the host plants, the most plausible explanation is that the fungus overwinters in the soil. As the Sclerospora oospores from bajra have now been germinated and their long viability proved, it would not be wrong to assume that in India the green-ear disease of bajra is perpetuated through the oospores, which are admirably suited to the purpose owing to their very resistant wall.

SUMMARY

Green-ear disease of bajra, caused by *Sclerospora graminicola*, has been studied. It causes considerable loss due to the reversion of the ear into a green, leafy condition. Unusual formation of green ear twice over in the

same plant has been noticed. No conidial stages have been found; only oospores are formed. Successful inoculation experiments, using oospores, have been performed. Though no green ears were formed on the inoculated plants, the leaves showed usual shredding with plenty of oospores. Following Hiura's method, bajra oospores have been germinated. These are found to remain viable even for 5 years. As zoospores are incapable of infecting bajra, this germination of oospores and their long viability and successful inoculation experiments with oospores show beyond doubt that the disease is propagated through the oospores in the soil.

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TRANSMISSION OF SMUT AND MOLDS IN FIGS

H. N. HANSEN AND A. E. DAVEY

During the past 3 decades the fig growers of California have suffered increasing losses due to various rots, decays, and fermentations of their product caused by cryptogamic organisms carried into the cavities of the figs by various insects. As early as 1892 Newton B. Pierce (10) stated: ". . . . the growers had to contend with a destructive fermentation of the fruit which often caused the loss of nearly the entire crop." And further: "The fruit is inoculated by insects." Howard (8) also suggests insect transmission of souring, while Condit (3) and Hodgson (6) mention a similar possibility in the case of the fungus infection called smut. Condit (4) states that souring is only another name for fermentation caused by the action of yeasts, molds, and bacteria. He attributes the introduction of these organisms into figs to insects, particularly "small beetles" and occasionally the vinegar fly. Phillips, Smith, and Smith (9), working particularly with "fig smut" (Aspergillus niger van T.), came to the conclusion that Carpophilus hemipterus L. and Drosophila ampelophila Loew. were the principal carriers of the causal organism. Caldis (1) has shown that Fusarium moniliforme var. fici Cald., which causes a rot (endosepsis) in caprified figs, is transmitted from the capri to the edible fig by the caprifying insect Blastophaga psenes L. The same author (2) also shows that Carpophilus hemipterus L. carries yeasts into the interior of both caprified and parthenocarpic figs. From this it will be seen that insect transmission of fig-spoiling organisms has long been recognized.

In speaking of insect transmission in connection with fig spoilage it has, however, been generally assumed that, so far as the parthenocarpic varieties of figs were concerned, no insects entered them until they were nearly ripe and the eye well opened (1, 9). In 1929 the senior writer published a short note (5) in which he shows that thrips enter green figs long before the eye scales loosen and bring into them various cryptogamic organisms capable of producing spoilage. Later (11) predaceous mites were observed in overwintering capri figs (mamme) and found to be carrying various cryptogams.

"Smut and molds" is the designation of one of the spoilage grades in dried figs set up by Howard (7) of the United States Food, Drug and Insecticide Administration. This grade includes all figs showing visible fungus growths in the interior, whether of the black, dusty type of smut (Aspergillus niger) or the variously colored mycelial growths of other fungi. The present paper deals with the presence of mites and thrips

¹ Unless otherwise specified, whenever mites are mentioned in this paper predaceous mites are meant.

in Adriatic (parthenocarpic) figs and their relation to the occurrence of smut and molds in these figs. Four places were selected near the figgrowing centers of the State (Madison, Modesto, Merced, and Fresno) from which representative samples of green, immature figs were obtained at intervals between July 1 and August 15, 1930 (Table 1). While the

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TABLE 1.—Showing seasonal variation of mites and thrips infestation in green Adriatic figs at Madison, California

Date	Number of figs examined	Percentage of figs harboring mites	Percentage of figs harboring thrips	Percentage of figs harboring thrips or both
July 1	100	29.0	11.0	36.8
9	100	18.0	4.0	21.7
" 15	200	18.5	8.0	25.0
" 19	350	18.2	9.4	25.9
23	200	25.5	4.5	28.9
" 31	200	27.0	2.5	28.8
August 4	200	25.5	6.0	30.0
8	100	34.0	5.0	37.3
15	100	21.0	0.0	21.0
Totals	1,550	23.2	6.1	27.2

fig is very young up to about the size of a large hazelnut, the eye scales are quite pliable, but, as it develops further, the scales become hard and rigid and are able to offer considerable resistance to any insect trying to enter the fruit. Later, as the fruit matures, the eye scales again loosen and spread apart until at full maturity there may be a clear passage to the interior of the fig from 2 to 5 mm. in diameter. It should be borne in mind that the fig tree produces its main crop on current-season wood and that new fruits are formed over the comparatively long period of from 4 to 6 weeks, so that figs in all stages of maturity may be found simultaneously on the same tree. In collecting our samples only green figs were taken and these in numbers representative of the various maturity stages from the hazelnut size up to the time when the eye scales begin to loosen again. The figs were taken to the laboratory at Davis, split into halves, and examined for mite and insect infestation by means of dissecting microscopes. attempts were made to determine the frequency of occurrence of individual species of mites and thrips or to determine numbers of mites and thrips in

TABLE 2.—Extent	of	infestation	of	mites	and	thrips	in	green	Adriatic	figs
			•							

Place	Number Place of figs		Figs showing predaceous mites		showing rips	Figs showing mites and thrips	
	examined	Number	Tumber Per cent Numb		Per cent	Number	Percent
Madison	1,550	359	23.2	95	6.1	422	27.2
Modesto	1,800	549	30.5	50	2.8	562	31.2
Merced	1,000	206	20.6	12	1.2	209	20.9
Fresno	920	268	29.1	24	2.6	282	30.6
Totals	5,270	1,382	26.2	181	3.4	1,475	28.0

individual figs. The data are summarized in table 2. During the progress of this examination mites and thrips taken from the interior of the figs were cultured on nutrient agar from time to time to determine the abundance and diversity of flora carried by them. (Table 3.)

TABLE 3.—Results of culturing mites and thrips from the interior of green Adriatic figs

	Number cultured	Number sterile	Percentage sterile	Number carrying cryptogams	Percentage carrying cryptogams
Mites	266	122	43.5	144	54.2
Thrips	194	78	40.2	116	59.8
Totals	460	200	43.5	260	56.5

In order to show the effect of maximum infestation of mites and thrips and, at the same time, exclude larger insects (mainly Carpophilus hemipterus and Drosophila ampelophila) from entering the figs, the following experiment was devised. During August 10 to 15 the still unopened eyes of 1,557 figs were effectively sealed by placing on the eye scales of each a

TABLE 4.—Effect of sealing the eyes of green Adriatic figs on the occurrence of smut and molds

	Number of	Figs with smut and molds		
Treatment	figs examined	Number	Per cent	
Sealed	1,557	359	16.6	
Unsealed controls	400	54	13.5	

small dab of tanglefoot preparation. Such treatment did not appear to injure the fruit in any way, as it developed and matured in normal manner and season. The treated figs were allowed to mature on the trees and were not collected until they had dropped to the ground, after which they were taken to the laboratory, split open, and examined for smut and molds. As control, 400 mature figs were picked from the ground under surrounding trees and examined likewise. (Table 4.)

DISCUSSION OF RESULTS

The percentages in table 1 show considerable variation of infestation of mites and thrips throughout the season but no considerable or consistent increase as the season advances. This indicates that the major part of infestation takes place while the fig is very young and the eye scales pliable.

Table 2 shows that mite and thrips infestation in green, immature figs is quite extensive and general throughout the State. It also shows considerable variation in amount of infestation between localities. Compare figures for Modesto and Merced. Annual variations (not shown in the tables) may, however, be much greater. For example, the table shows thrips infestation for Madison to be 6.1 per cent, whereas thrips infestation at the same place, the previous year, was over 18.0 per cent. The factors responsible for these variations are not evident.

Table 3 shows that more thrips than mites are carriers of cryptogams. This is probably due to the greater size of the insects. Since nearly half of the mites and thrips found in figs are sterile, direct prediction as to the amount of loss to be expected from smut and molds can not be made on the basis of the percentage of figs infested. However, an indicative figure may be obtained by multiplying the percentage of figs infested by that of mites and thrips carrying cryptogams. For example, the total percentages in tables 2 and $3\frac{28.0 \times 56.5}{100}$ gives 15.2 per cent, a close approximation to the percentage of actual smut and mold loss shown in table 4.

The data given in table 4 indicate strongly that the major part of smut and mold loss is due to cryptogamic organisms carried into the green figs by predaceous mites and thrips long before the eye scales begin to loosen, and they clearly show that the presence of Carpophilus hemipterus and Drosophila ampelophila is not at all necessary for the occurrence of this type of spoilage. The higher percentage of smut and molds in the figs with sealed eyes probably can be attributed to closing of the eye, which might be expected to create a more humid condition in the fig, favoring germination of mold spores.

The cryptogamic flora in green and ripe figs and on mites and thrips, cultured, included the following species named in the order of the fre-

quency of their occurrence: Miscellaneous fungi, bacteria, *Hormodendrum* spp., *Aspergillus* spp., *Penicillium* spp., *Alternaria* spp., *Rhizopus* spp., *Acrostalagmus* sp., and a few yeasts.

FAUNA

The mites found in green figs consisted of Sejus pomi Parrott, Cheyletus, sp., and two other unidentified species. The mites are very small, varying in length from 0.4 to 0.9 mm. They have been observed to feed on the common fig mite, Eriophyes fici Ewing. These predaceous mites usually confine their activity to the eye region of the fig and are seldom found deep in the interior. The common fig mite was found in about 90 per cent of all figs examined. It is apparently insignificant as a carrier of cryptogamic organisms but may be of considerable importance in attracting predaceous mites. Of thrips, the following were found: Frankliniella tritici (Fitch), F. tritici var. californicus (Moulton), Thrips bremneri (Moulton), Liothrips ilex (Moulton), Heliothrips fasciatus (Perg), and the predaceous thrips, Leptothrips mali (Fitch). All of the mites listed and at least 2 of the thrips, F. tritici and Leptothrips mali, have been found to breed within the figs of all crops, both edible and capri. These thrips, with the exception of mali, feed upon the tender floral parts of the fig. They are thus not confined to the eye region, as are the mites, but wander throughout the interior of the fig and, for this reason, they are probably more effective as carriers of spores.

SUMMARY

Some of the literature relating to transmission of fig-spoilage organisms is reviewed.

It is shown that figs are entered by mites and thrips long before the eye scales begin to loosen.

It is demonstrated that mites and thrips are carriers of cryptogamic organisms capable of producing smut and molds in figs.

It is proved experimentally that presence of the larger insects Carpophilus hemipterus and Drosophila ampelophila is not necessary for occurrence of smut and molds in figs.

The list of the mites and thrips and the fig-spoilage organisms they carry is appended.

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SEED DISSEMINATION IN FUSARIUM WILT OF PEA1

W. C. SNYDER²

INTRODUCTION

It has been only since the paper of Linford (5), in 1928, that Fusarium wilt of pea has been recognized as a major disease of the garden and canning pea, *Pisum sativum* L., in the pea-seed and canning areas of the United States. Within the past 3 or 4 years the disease, attributed by him to *F. orthoceras* App. and Wr. var. *pisi* Linford, has been found in at least 10 States extending from Washington to Maryland (6).³ The sudden occurrence of wilt in widely separated pea-growing areas not previously known to harbor the disease, and other field evidence, have suggested the possibility of dissemination with the seed, although it is common knowledge that local dissemination occurs by means of diseased vines, implements, water, and other agencies of soil transfer from wilt-infested fields.

In Linford's 1928 survey, wilt was found for the first time in Montana and Idaho, where the bulk of the pea seed for the United States is grown. It appears significant that in both States foci of infection included the trial grounds of large seed companies. A less extensive survey by J. C. Walker and the writer, in 1930, showed the practical limit of infestation in this area was centered in the trial grounds. In the case of both States there has been a free exchange of seed lots with other States where wilt is known to occur, which points to strong circumstantial evidence in favor of seed transmission.

An attempt, therefore, has been made during the past year to definitely ascertain whether the disease may, under certain conditions at least, be transferred to clean soil with the seed. Results so far obtained are discussed in the body of this report.

Other related vascular diseases have been shown to be seed-borne. Bolley (1) reported that flax wilt may be spread by way of the seed, spores of the fungus being lodged upon the surface of the seed with dust at the time of threshing. Elliott and Crawford (3) have presented both field and laboratory evidence on the seed-borne occurrence of tomato wilt. They were able to follow the progress of the fungus up the vascular bundles

¹ Published with the permission of the Director of the Wisconsin Agricultural Experiment Station.

² Grateful acknowledgment is made to Dr. J. C. Walker under whose guidance the work has been carried out.

³ Linford, Maurice B. Pea diseases in the United States in 1928. U. S. Dept. Agr., Bur. Plant Indus., Plant Disease Rptr. Sup. 67. 1929. (Mimeographed.)

of the plant into the pedicels and fruit. Tomato fruits so infected were reported to have $3\frac{1}{2}$ per cent of their seed infected after a 7-month period, a smaller percentage of which harbored the fungus within the seed coats. Elliott (2) was able also to isolate the cotton-wilt fungus from the seed of infested bolls where, again, it was recoverable both on the surface and in the seed coats. In the case of pea wilt, however, the invading fungus is generally limited to subterranean portions of the plant entering only the lowest internodes of the aboveground plant. Linford (5) states "in no case was the fungus found within two internodes of the lowest pod." He removed asceptical seed from pods of wilted plants, plating them in Petri dishes of agar, but failed to obtain any trace of the parasite. These observations indicated that dissemination of wilt with pea seed occurs very rarely, if at all, and check with field experience where only an occasional wilt spot may be seen to occur in a several-acre field at the time disease is first observed to be present.

METHODS

In the summer of 1929 vines of susceptible varieties of pea, growing on wilt-infested soil, were collected in cases where some seed had been set before wilting was completed. Each internode of these plants from the ground upward was cultured for the wilt fungus and its position in relation to pod nodes carefully noted. In every case save one Linford's experience was duplicated. In the single exception out of about 50 plants the fungus was isolated from the stem internode immediately below the attachment of the lowest pod. The extreme limit of invasion, however, was confined, even in this case, only to the stem, for platings of both pedicel and seed of the lowest pod failed to give any trace of the pathogen.

A different approach was attempted the following year in order to test large populations of seed. The plan consisted in harvesting in bulk the seed occasionally set in the field by wilted plants, and sowing them in successive series upon wilt-free soil in the greenhouse. By this means the accumulation in the soil of small amounts of inoculum could be followed to the point of disease inception. Steamed soil was not used because of its inhibiting effect on early establishment of the fungus. The procedure was as follows:

A bench $3\frac{1}{2} \times 12$ ft. was selected in a section of greenhouse that had not previously been used for peas and was away from the main aisle. The bench was thoroughly washed, then repeatedly drenched with 1 to 400 hot corrosive sublimate. Virgin soil, far removed from cultivated fields, was obtained from beneath the turf on a knoll in Madison. There is every reason to believe it was free of the wilt organism, similar soil having been used repeatedly for healthy controls in inoculation work during the past

year with never a single case of wilt. No implement, conveyor, or article touched the soil from the time of its removal until its spreading in the bench, unless it was sterilized and every precaution was taken in planting, watering, and manipulating the bed to prevent any chance contamination with wilt. The opportunity for this was remote under these circumstances, especially since the organism bears few conidia. In this bed of soil was planted seed harvested from diseased plants collected in the summer of 1930.

The seed used was obtained from two different wilt fields in Wisconsin, one at Columbus, where the wilted plants in a 50 per cent resistant Alaska stock were pulled and the seed threshed, and the other at Beaver Dam, from small plots of Ashford and Acme (both completely susceptible varieties), which had been planted for a canning test but were lost through Fusarium wilt.

Beginning with September of 1930, 4 successive plantings were made using the seed described above, seeding the first 2 times with the Alaska seed, and the third and fourth times with a mixture of the Ashford and Acme seed, as indicated in table 1. The seed was thickly planted both in order to use large numbers of seed and because of a high percentage of nonviable, shriveled peas. This brought the total seed population planted in the bed from diseased plants to 7,900. A fifth planting of seed of the susceptible variety Perfection was sown at the conclusion of the tests. This last lot of seed was produced on wilt-free soil.

Each planting was allowed to remain in the bed for a period of 7 weeks, and a soil temperature was maintained within the optimum range for wilt development between 18° and 24° C. Under these conditions, on naturally infested soil, wilting normally occurs within 3 to 5 weeks. Upon the appearance of disease symptoms plants were pulled and cultured for positive identification of the pathogen. At the end of each series all plants were pulled and examined macroscopically.

RESULTS

In the first 3 crops, as shown by table 1, no symptom of wilt appeared. In the fourth 4 separate plants at different points on the bed showed distinct symptoms of wilt, both aerially and subterraneously and, on being cultured, each yielded colonies of the pea-wilt Fusarium. A pathogenicity test of these cultures, using Perfection seed upon artificially inoculated soil, proved their authenticity in each case.

From the fifth seeding with Perfection harvested from a wilt-free area, a few suspicious plants were cultured before the plants dried up from extreme heat. Of these, 3 were found to be infected and *Fusarium orthoceras* var. *pisi* was recovered from them and used successfully in further patho-

genicity tests. It is probable others might have been found had this run gone to completion, but the 3 obtained suffice to demonstrate clearly that the wilt fungus had become established in the soil.

TABLE 1.—Appearance of wilt in successive plantings of seed from pea plants infested with Fusarium orthoceras var. pisi

Series	Variety	Source of seed	Number of seeds planted	Number of wilted plants	
1	Alaska	Columbus, Wisconsin	1,850	0	
2	"	"	1,850	0	
3	$\left\{ egin{array}{l} ext{Ashford} \ ext{Acme} \end{array} ight.$	Beaver Dam, Wisconsin	2,100	0	
4	a.		2,100	4	
5	Perfections	Idaho	2,000	3b	

² The seed used in the fifth series was grown on wilt-free soil.

DISCUSSION

These results, supported by field observations of both Linford and the writer, taken over 2 periods of years, are interpreted as definite evidence that the Fusarium wilt of pea may be occasionally, though not abundantly, carried with the seed. They do not reveal the manner in which the fungus is carried, whether within the seed coats or upon the surface as spores adhering to the seed coats in dust or associated with soil particles and other bits of refuse attending the seed.

The conditions of these trials should be taken as a greatly exaggerated field condition in that only seed from wilted plants was used. Yet, out of a population of about 8,000 seeds, selected for their greater possibility of carrying infection, only 4 foci of infection were established in the seed bed. These first 4 cases of wilt may be interpreted as the accumulative effect of small amounts of inoculum introduced with the various series and not necessarily the result of inoculum brought in with only the fourth seeding.

It is common observation that even in factory-cleaned seed, an occasional pea seed, especially of the wrinkled types, may be found with a small piece of soil lodged in an indentation of the surface. Since the fungus produces chlamydospores abundantly, it is plausible to conceive of the fungus being carried in this medium. In addition, since the fungus is occasionally found in the stem node giving origin to the lowest pod of a wilted plant, it is likely that once in a great while invasion of the pedicel and thence

b Incomplete reading owing to death of the plants from extremely high temperatures.

of a seed may take place. Invasion of the seed may also occur in an additional manner where the pods lie near or upon the ground. Jones (4) in testing pea seed for the presence of Ascochyta spp. isolated from surface-sterilized seed various fungi including Fusarium spp. as well as Ascochyta spp. and bacteria. Since other Fusarium spp. are shown to be present occasionally in the interior of pea seed, there seems to be no reason why F. orthoceras var. pisi should not also enter in this manner, since it grows as a saprophyte on the plant refuse and may well be expected to follow active pod invaders or even directly enter maturing pods in contact with the soil.

The establishment of seed transmissibility of the Fusarium-wilt organism is of little concern from the practical standpoint in regions where wilt is already present, but it does confirm a long-held opinion of those in contact with the wilt problem. Seed treatment on a commercial scale to catch the rarely infected seeds would be impractical and uneconomical and with the increasing use of wilt-resistant varieties (6) in the important wilt areas, it will be of even less concern. However, it is clear that pea seed from wilt fields is a potential source of primary inoculum for any wilt-free soil suited to the establishment of the fungus.

SUMMARY

The organism causing the Fusarium wilt of pea is shown to be occasionally carried with pea seed harvested from wilt-infested land. This means of transmission, while relatively rare, is, nevertheless, to be regarded as a potential source of primary inoculum whereby the organism may be introduced into non-infested soil.

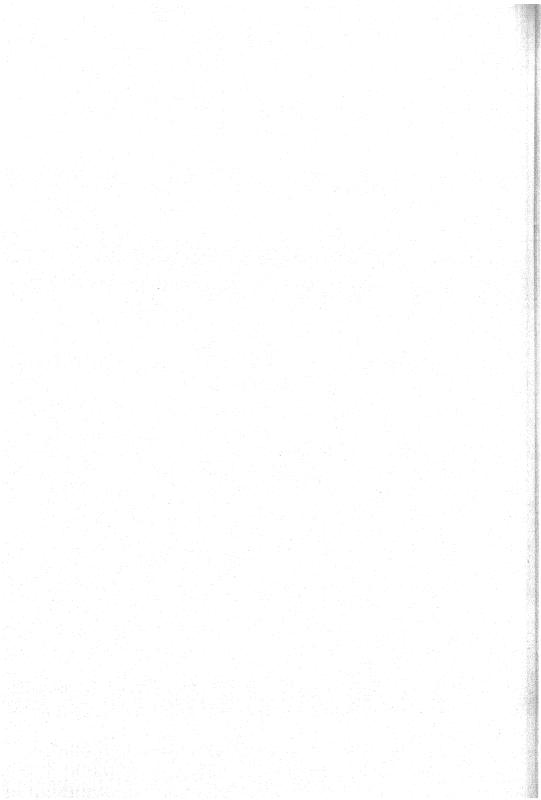
The exact manner in which the fungus is carried is not known.

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EFFECT OF BUNT ON HEIGHT OF WHEAT PLANTS¹

E. N. BRESSMAN

Bunt, or stinking smut, of wheat includes two species, *Tilletia tritici* (Bjerk.) Winter and *Tilletia levis* Kühn. *Tilletia tritici* is frequently referred to as "low smut" and *T. levis* is called "high smut," because of the height of the bunted culms in comparison with normal ones. In 1918 Potter and Coons² confirmed the earlier observations of a Michigan farmer that there were two types of infected plants, "high" and "low."

Because of the common belief that the species differ in the above way, record was made of the relative heights of the diseased and normal plants in the writer's 1928 tests at both Moro and Corvallis, Oregon, conducted to determine physiologic forms of bunt. There were 430 rows from 8 to 10 ft. in length at each place. The seed of 10 varieties of wheat, Hybrid 128, Albit, Ridit, Oro, Turkey × Bearded Minnesota 48, Hussar, Regal, Martin, White Odessa, and Banner Berkeley, was inoculated with 43 collections of bunt spores. Details of the procedure and the sources of these collections are published elsewhere.³

At the time of making smut counts notes were taken on the relative height of the bunted plants as compared with noninfected plants. The results are tabulated in table 1. The estimates at Moro, Oregon, were made by J. Foster Martin.

A summary of the classification of the height of bunted plants shows that of the 387 rows of *Tilletia levis*, 293 were classified as low and 94 were called normal or low to normal, which is a ratio of about 3 to 1. Of the 223 rows of *T. tritici*, 194 were classified as low or low to normal and 29 normal, which is a ratio of 6.7 to 1. The *T. levis* classification on a 6.7 to 1 basis gives a value of 25.67 for X^2 . This means that the odds are several thousand to one that the difference in height of smutted culms thrown by these two species is not due to chance alone.

On a 3:1 basis the 6.7:1 ratio has a P. E. of 5.2. The deviation is 26.75 and deviation divided by P. E. is 5.1 or odds of more than 1,000 to 1 that the two species are different in height of smutted culm.⁴ Both

- ¹ Published as Technical Paper No. 155 with the approval of the Director of the Oregon Agricultural Experiment Station, a contribution of the Department of Farm Crops.
- ² Potter, A. A., and G. W. Coons. Differences between the species of the Tilletia on wheat. Phytopath. 8: 106-113. 1918.
- ³ Bressman, E. N. Varietal resistance, physiologic specialization, and inheritance studies in bunt of wheat. Oreg. Agr. Exp. Sta. Bul. 281. 1931.
- ⁴ Hayes, H. K., and R. J. Garber. Breeding crop plants. 438 pp. 2nd Ed. McGraw-Hill, New York. 1927.

T-Tilletia tritici TABLE 1.—Relative height of bunted heads in 10 varieties of wheat, inoculated with 43 collections of bunt. and L-Tilletia levis. Corvallis (C) and Moro (M), Oregon, 1928

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These ^a la, lb, etc., are collections of bunt.

^b L is lower than normal culm. N is same height as nomal culm. LN varies from lower to same height as normal culm. are classified as L or N, depending upon species. Where no culm height is indicated, no bunt occurred.

TABLE 1.—Relative height of bunted heads in 10 varieties of wheat, inoculated with 43 collections of bunt. T-Tilletia tritici and L-Tilletia levis. Corvallis (C) and Moro (M), Oregon, 1928

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^a 1a, 1b, etc., are collections of bunt.

^b L is lower than normal culm. N is same height as nomal culm. LN varies from lower to same height as normal culm. These are classified as L or N, depending upon species. Where no culm height is indicated, no bunt occurred.

species tend to produce plants lower than the normal. But, the foregoing analysis shows that, on the average, the *Tilletia levis* infected plants are taller than those infected with *T. tritici*. If the rows with smutted plants of varied height were not considered, the difference would not be so great.

Hybrid 128 produced taller and more varied infected plants than did the other varieties in the trial. Of the 52 *T. levis* infected Hybrid 128 rows, 13 were classified as low and 39 as normal or low to normal. Of the 34 *T. tritici* infected Hybrid 128 rows, 25 were classified as low or low to normal and 9 as normal. On the other hand, practically all of the smutted Banner Berkeley plants were low.

Collection 1e is of special interest, as it comprised several heads of Tilletia tritici produced on plants that were as tall as those of nonsmutted plants. It was collected at Corvallis from what has come to be known as "Palouse" bunt. This collection was obtained in the fall of 1926 from the office of Federal Grain Supervision, Bureau of Agricultural Economics United States Department of Agriculture, Oregon. The original material is the same as that used by D. E. Stephens at Moro, Oregon, and mentioned by Gaines as showing striking results at Moro. Collection 1e did not continue to show these tall bunted plants in 1927-28. This indicates that environmental conditions, as well as the species, may affect the height of the smutted plants. Also, the results with this collection may account for the variation in height of smutted plants found in certain rows. It is of interest to note that both collections of 1d and 1e are classified by the writer as physiologic form 7 because they are similar in their smutting of the differential hosts. Collection 1d consisted of the low infected plants and 1e the high infected plants found in Palouse bunt.

In general, *Tilletia levis* produces a taller infected plant than *T. tritici*, but it is not a constant characteristic of the species. Varieties and environmental conditions also have an effect on the height of the infected plants. There appear to be other differences of greater importance within the species than between the two.

OREGON AGRICULTURAL EXPERIMENT STATION, CORVALLIS, OREGON.

⁵ Gaines, E. F. New physiologic forms of *Tilletia levis* and *T. tritici*. Phytopath. 18: 579-588, 1928.

PHYTOPATHOLOGICAL NOTES

An atypical lesion on cotton leaves caused by Bacterium malvacearum.—In the summer of 1929 the writer received from O. C. Boyd some cotton leaves collected in North Carolina, with a query as to the cause of the large dead areas. He reported that this type of lesion was causing more serious defoliation than the usual angular spot.

The spots, marginal or otherwise, 1 to 3 cm. wide, irregular in shape, appeared to be the result of vascular rather than stomatal infection, formed by a gradual and progressive fading out and dying of the tissues; when marginal, advancing from the margin inward. In the pale green or gray areas the veins were darkened, not with the well-known external blackening occurring in typical angular spot, but with such as results from an internal disturbance (Fig. 1).

Sections cut across such lesions show a heavy infection in the vascular system. Bacteria pour out in great numbers from the cut ends of the veins but not from the intervening tissue. This is not true of typical angular spot, from which the bacteria are extruded from all of the tissues of the entire area of the spot. Bacteria were found in the veins at some distance from the discolored area and in one instance were plated from the petiole. In no case, however, was it found that the bacteria had entered through the petiole. They had spread from an infection on the blade.

The point of entry, whether by a water pore or through an inconspicuous lesion on a vein, as sometimes appears to be the case, has not been determined. It seems evident, however, that this strain of the organism is able under some conditions to enter and spread through the vascular system.

Plates were poured and a yellow bacterial organism obtained that resembles *Bacterium malvacearum*. Inoculations made by spraying cotton leaves in the hothouse, using subcultures from these isolations, gave typical angular-spot lesions on leaf blades, petioles, veins, and bolls, as well as large, pale spots like those from which the culture used was obtained. Reisolations and repeated inoculations with reisolations have given the same results.

Parallel inoculations with a freshly isolated strain of Bacterium malvacearum from typical angular spot from another source gave the typical angular lesions but none of the systemic infections. No record has been found of the above type of spot, nor has the writer ever obtained it before in numerous greenhouse inoculations made in the past with Bact. malvacearum from various sources. Since only minor cultural differences have been found between this strain and strains from typical lesions, it seems

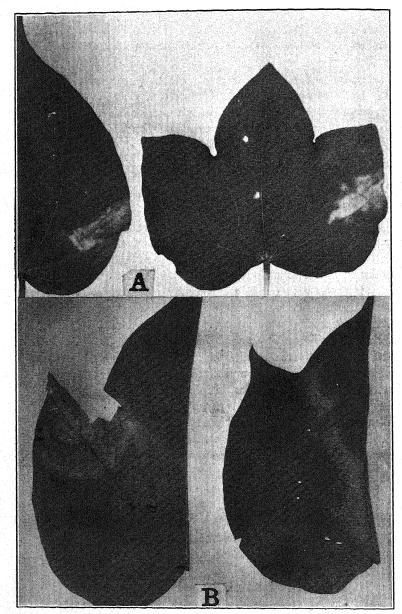


Fig. 1. A. Young spots produced by inoculation with the North Carolina strain of Bacterium malvacearum. B. More advanced stage, showing darkened veins.

evident that the organism in question is *Bact. malvacearum*.—Mary K. Bryan, Bureau of Plant Industry, United States Department of Agriculture.

Root, crown, and shoot rot of milo.—In the Southwestern States, where grain sorghums are grown extensively, milos have, in some localities, been showing a marked susceptibility to a certain root, crown, and shoot rot. On certain field experiment stations the disease has appeared first on plots continuously cropped to milo for a number of years or on plots where there have been relatively short intervals between crops of milo. In the more severe cases it has caused complete failure of the crop and is becoming a limiting factor in milo production.

Dwarfing of the plants and lack of heading are common symptoms of the disease. (Fig. 1.) Where the disease is complicated by chinch-bug in-

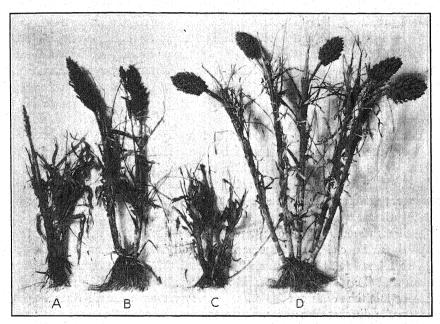


Fig. 1. A, Diseased, and B, healthy plants of Custer milo; and C, diseased, and D, healthy plants of Dwarf Yellow milo. Garden City, Kansas, 1930.

jury, there is an abnormal amount of tillering. Where there are no chinch bugs, plants may grow 10 to 12 in. in height before any symptoms of the disease appear. The lowest leaves turn yellow along the margins, the yellow color gradually spreading over the entire leaf and appearing on the leaves above. The plants remain stunted and produce no heads, or only small ones, and a gradual drying and decay of the plants follow. These symptoms above ground are usually accompanied by a dark red discoloration of the central cylinder of the roots and of the interior of the crown. This red discoloration may spread throughout the crown and up into the

lower nodes of the stalk and is often followed by a brown discoloration and decay of roots and crown. In some cases there is decay of the central shoot with or without the other symptoms.

These symptoms have occurred under different soil and climatic conditions of southeastern, south-central, and northwestern Texas, northwestern Oklahoma, northeastern New Mexico, western Kansas, and southeastern California.

The following facts point to the parasitic nature of the disease: Both susceptible and resistant varieties of sorghum have shown few or no disease symptoms when grown in soil where the disease has not been previously evident. Susceptible varieties grown in soil that had produced the disease previously have shown 100 per cent diseased plants, while resistant varieties in the same soil have shown few or no disease symptoms. When such soil is sterilized by steam under pressure or by treatment with formal-dehyde, both susceptible and resistant varieties have grown normally and have remained healthy. Preliminary tests have indicated that the disease symptoms may be produced by the addition of infested soil or diseased roots to the sterilized soil.

While all of the western soils tested have been found to be more or less alkaline, the most alkaline, tested by the quinhydrone method, was pH 7.41. By the LaMotte method the reading was pH 8.0, both for the clean as well as the disease-producing soil. Analysis of the infested soil showed no excess of total soluble salts such as might be concerned in the production of disease symptoms.

Soil treatments with potash, phosphates, nitrates, and complete fertilizers so far have shown no indications of soil deficiencies.

Greenhouse tests with infested soils from three different localities have shown that the plants react somewhat differently in the different soils, and it is possible that different factors may be responsible for the disease symptoms in the different localities.

A white bacterium and strains of Fusaria have been consistently isolated from diseased plants from different localities, but, to date, pureculture inoculations have not demonstrated the pathogenicity of any of these organisms.

Varietal tests conducted at Garden City, Kansas, have shown that all typical milo varieties are very susceptible to the disease in question and that most milo hybrids are more or less susceptible. Kafirs, feteritas, sorgos, and Fargo Straightneck milo (a kafir-milo hybrid) are resistant. Studies are being conducted cooperatively by the Division of Cereal Crops and Diseases, Bureau of Plant Industry, the Kansas Agricultural Experi-

¹ Electrometric pH determinations and soil analyses were made by the Bureau of Chemistry and Soils, United States Department of Agriculture.

ment Station, and the Texas Agricultural Experiment Station.—Charlotte Elliott, Bureau of Plant Industry, United States Department of Agriculture; F. A. Wagner, Garden City Branch of the Kansas Agricultural Experiment Station, Garden City, Kansas; and L. E. Melchers, Kansas Agricultural Experiment Station, Manhattan, Kansas.

Sulphur dioxide injury of tomatoes.—For several years occasional cars of tomatoes showing deeply sunken areas about the stem end have been observed on the markets. The tomatoes in most cars appeared normal in every respect except for the sunken stem end and a bleached greenish tan discoloration of the immediately surrounding tissues. (Fig. 1). Prelimi-

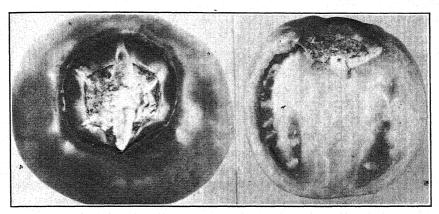


Fig. 1. Stem-end and longitudinal sectional views of a tomato showing sunken cracked areas and bleached surrounding tissues.

nary investigations indicated that the injury was not due to a parasite but was possibly induced by some unfavorable environmental condition.

The similarity of this injury to that caused by fumigation of water-melons with formaldehyde¹ suggested the possibility that the tomatoes were affected by some gas. Laboratory experiments demonstrated that this type of injury could be produced by exposing tomatoes in a closed container to the fumes from burning sulphur. Later investigations of certain cars of tomatoes showing the symptoms under consideration established the fact that the fruits had been subjected to fumigation by burning sulphur in the loaded cars at shipping point.

Tomatoes injured by exposure to sulphur dioxide gas have been observed on the markets in stock from California and Florida. Although only a few cars have been found showing this type of trouble, the serious-

¹ Ramsey, G. B. Fumigation injury of watermelons. Phytopath. 25: 479-481. 1925.

ness of the injury warrants mention. It may result from burning sulphur in cars of tomatoes or using sulphur dioxide gas, as is practiced with certain commodities in California, to reduce the amount of decay during transit and marketing. However, it appears that no experimental data are available to show that this treatment is of value in reducing decay in cars of tomatoes.

The affected tomatoes show a very pronounced sinking and drying out of the tissues at the stem sear. The gas enters the fruits through this sear or through wounds elsewhere on the surface and the cells immediately surrounding the openings are killed and lose their water rapidly by evaporation, causing collapse of the affected regions. In some instances the stem scar is sunken 1 inch or more and the tissues around the scar are bleached a sickly greenish tan color, while the remainder of the tomato retains the normal color except where mechanical injury is evident. Wherever the cuticle and epidermis of a fruit show a wound, the surrounding tissues are sunken and bleached, like those around the stem end. These sunken areas dry and crack, thus opening the way for various secondary decay-producing organisms, such as Alternaria, Rhizopus, and Fusarium. Decay induced by these fungi becomes especially damaging in the greener fruit that is held under the humid and high-temperature conditions of the ripening rooms on the receiving markets.—G. B. RAMSEY. Division of Horticultural Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture.

Black-walnut canker caused by a Nectria.¹ In a former issue of Science, Orton² reported the occurrence of a canker of black walnut, Juglans nigra L., in West Virginia. A survey of the State, begun early in the spring of 1930, has traced this disease in 24 counties extending from the northern to the southern boundary line of the State. The canker has been located also in Virginia and Pennsylvania and reported from Tennessee, Rhode Island, Wisconsin, and Ontario.

The frequent association of the red perithecia of a Nectria with the canker led to the assumption that this organism might be the causal agent. Consequently, series of inoculations were made at intervals of 2 weeks, beginning in January and ending in May.

All inoculations were made outdoors on the limbs and branches of older trees, as well as on the trunks of young ones, 2 to 5 years old. Conidia from pure cultures obtained from ascospores of the Nectria were used for

¹ Published with the approval of the Director, West Virginia Agricultural Experiment Station, Scientific Paper No. 99. The support of a David Allan Burt Fellowship in West Virginia University is also acknowledged.

² Orton, C. R. Black walnut canker. Science, n. s. 72: 142-143. 1930.

inoculum. The inoculations were made by puncturing the bark with the point of a sterile scalpel and inserting the inoculum in the wound, which was then wrapped with cotton and adhesive tape. Check wounds were treated in the same manner, except that no inoculum was inserted.

Of the 104 inoculations made between February 18 and April 28, inclusive, 82, or 79 per cent, showed a sunken area corresponding to the early stages of the canker. The results of the inoculations made in January could not be determined with certainty. This may have been due to the fact that the inoculations were made on large limbs on which the early stages of the disease do not show so readily, or that the temperature at the time the inoculations were made was too low for successful infection (-5° C.) .

Of the 58 checks, 53, or 92 per cent, were undoubtedly free of infection. The other 5 showed a slight necrotic area that either was a local irritation or was caused by a natural infection occurring too late in the season to have assumed any size.

The fungus has been repeatedly reisolated from these artificially induced cankers.

The most noticeable character of the infections at this early stage is the sunken appearance of the dead area. This condition is due to the fact that, with the resumption of cambial activity, a new layer of wood is formed around the dead area by the noninfected cambium. The infection spreads more rapidly in the direction of the long axis of the stem, thus appearing more or less spindle-shape in outline. On 1- or 2-year old branches, which are normally light brown, the necrotic tissue may be further marked externally by a change to a dark brown. Scattered, tiny, whitish pustules protrude through the dead tissue. These consist of the sporodochia of the fungus. They appear most frequently at the lenticels, since these form the most natural breaks in the dead bark. Observed under the microscope, the sporodochium is seen to consist of a cushion-like stroma, from the outer, rounded surface of which the cylindrical conidia radiate in great numbers.—J. M. Ashcroft, Department of Plant Pathology, Agricultural Experiment Station, Morgantown, W. Va.

Botrytis stem infection in pears. In a study of Botrytis rot of pears on fruit stored at Hood River, Oregon, it was found that certain lots of the 1930 crop showed a very high percentage of stem infection. The use of chemically treated paper wrappers to prevent the spread of Botrytis rot has made it easy to determine the number and place of initial infections.

Stem infection is characterized by the blackened tip of the stem having a definite line of demarcation between the healthy and the sound tissue. The diseased portion of the stem is usually soft and water-soaked and readily crumbles when rubbed between the thumb and finger. As the

storage season advances the discoloration moves down the stem and finally the fungus reaches and invades the fruit. It is unwise to try to hold fruit having infected stems until the late market, for the infection spreads from one fruit to another so rapidly that several fruits soon become rotten at each locus of infection.

The stem-infection type of rot was very sporadic on the 1930 crop in Hood River Valley. The fruit from most of the orchards showed very little of it, but that from certain orchards showed such severe stem infection that repacking was necessary because of rots resulting from the stem infection. Some boxes from the severely infected lots showed as high as 18 per cent stem infections. The lots showing severe stem infection came from orchards bearing a heavy cover crop of vetch or clover. Since dead and dying stems and leaves of such cover crops are known to be a favorable substrate for the growth and sporulation of Botrytis, an abundance of air-borne spores is probably present at pear-harvest time and available for inoculation of the abscissed stem ends of freshly picked pears.—J. S. Cooley, Bureau of Plant Industry, United States Department of Agriculture, Washington, D. C.

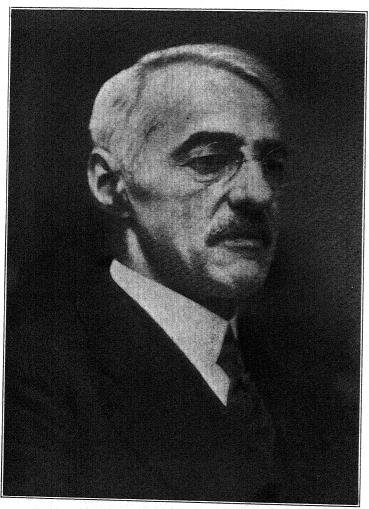
Black rot of stocks. In the spring of 1929 and, again, in the spring of 1930 a wilt was prevalent among stock plants (Matthiola incana R. Br. var. annua Voss) in floral greenhouses in the vicinity of Knoxville, Tennessee. A bacterium was isolated from affected plants and its pathogenicity was proved by inoculations in wounds. In its morphology and physiology the organism resembled Bacterium campestre (Pam.) E. F. Smith, the pathogen causing black rot of crucifers. Moreover, the symptoms of the disease in stocks were similar to those produced by the black-rot organism in other crucifers.

Von Faber reported Bacterium campestre as causing black rot of Matthiola incana R. Br. in Germany in 1907¹. From von Faber's paper Elliott, in her recent book, lists M. incana as a host of Bact. campestre.² Being unable to find any other report of black rot of stocks in the literature, the writer considers this probably the first record of Bact. campestre on Matthiola species in the United States.—L. M. Cooley, Department of Botany, University of Tennessee, Knoxville, Tennessee.

¹ von Faber, F. C. Über eine Bakterienkrankheit der Levkoyen. Arb. K. Biol. Anst. Land- u. Forstw. 5: 489-492. 1907.

² Elliott, Charlotte. Manual of bacterial plant pathogens. ix + 349 pp. The Williams & Wilkins Company, Baltimore. 1930. (See page 100).





CALVIN HENRY KAUFFMAN 1869-1931

PHYTOPATHOLOGY

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CALVIN HENRY KAUFFMAN 1869–1931

E. B. MAINS

Calvin Henry Kauffman was born March 1, 1869, on a farm in the little community of Kimmerling's near Lebanon, Pennsylvania. He died June 14, 1931, at the age of 62, at his home in Ann Arbor, Michigan, following an illness of 16 months' duration. He came from a long line of sturdy Pennsylvania German ancestors who settled in this country in the early days.

Doctor Kauffman received his early schooling in the little country school adjacent to the Kimmerling's church. As a young man he took an active part in the social life of the community. His musical talents found expression in playing the church organ and in directing singing schools in the neighborhood. At the age of 18 he began his career as a teacher and, for 3 years (1887–1890), taught in the secondary schools of Lebanon.

In 1890 he entered the Palatinate College, Myerstown, Pennsylvania, where he spent 2 years preparing for entrance to Harvard University. In 1892 he matriculated at Harvard and the 4 succeeding years were spent there. He specialized in languages and was graduated with distinction in 1895 with an A.B. degree. In 1895 he married Elizabeth Catharine Wolf, by whom he is survived.

Following his graduation he held the position of principal in a secondary school in Lebanon for 2 years (1896–1898). He then taught for 2 years (1898–1900) in a high school at Decatur, Indiana, and the following year in a normal school at Bushnell, Illinois.

Although he devoted but little of his time to science while in the University, he very early showed a special interest in botany. Before entering Harvard he, independently, made a study of the flora about Lebanon and started his herbarium of flowering plants. It was at Bushnell that his interest was turned definitely into the field of science. This was instigated partly by teaching a course in chemistry. He delighted in telling of a successful laboratory that he constructed from an old kitchen. While at Bushnell he acquired a copy of Atkinson's "Mushrooms" and spent considerable time studying the agaries of the vicinity.

As a result he decided to train himself in science and the following year (1901–1902) he spent at the University of Wisconsin where he came under

the influence of Professor R. A. Harper, who first aroused his interest in the Phycomycetes. The next year he went to Cornell University as a personal assistant to Professor G. F. Atkinson. Here he had as fellow students H. H. Whetzel, H. S. Jackson, J. M. Van Hook, and Charles Thom. For 2 years (1902–1904) he studied with Professor Atkinson and became keenly interested in the study of agarics.

In 1904 he accepted an instructorship in the botany department of the University of Michigan. He threw himself enthusiastically into his mycological studies and continued his field collecting as he had been doing so thoroughly at Cornell. He renewed his studies of a problem concerning the physiology of Saprolegnia species, which he completed and presented for his doctorate thesis in 1907. The dissertation was entitled "A Contribution to the Physiology of the Saprolegniaceae with Special Reference to the Variations of the Sexual Organs." Through the use of proper nutrient media he was able to control with remarkable accuracy the development of the species studied and change them from vegetative to reproductive phases, at will. In this investigation he became acquainted with the work of George Klebs, with whose theories his results agreed. Throughout the rest of his life the Klebsian principles dominated his own physiological studies and those of his students.

While at Cornell he became interested in the difficult genus Cortinarius, and in 1905 he published his first paper on the agarics, entitled "The Genus Cortinarius, a Preliminary Study." During these studies his attention was attracted to a mycorrhiza caused by Cortinarius rubripes Kauff., and he published his observations in a paper entitled "Cortinarius as a Mycorrhiza-Producing Fungus." The interest in mycorrhiza thus aroused was always maintained and was communicated to many of his students in forest pathology. For several years he devoted his attention to taxonomic studies of fungi of Michigan with special reference to the Agaricaceae. His field work took him to many parts of the State. Studies based upon these collections were published in a series of papers that appeared from year to year in the Michigan Academy Reports under the title "Unreported Michigan Fungi." His studies in agarics resulted in the publication in 1918 of a work of 2 volumes, "The Agaricaceae of Michigan," which contains critical descriptions of 884 species and is illustrated with 172 plates. This work may be classed as one of the major contributions to the knowledge of the Agaricaceae in this country.

The summer of 1908 he spent in Europe acquainting himself with the agaries of northern Europe.

In 1914 he spent a very profitable summer collecting and studying fungi in Peck's favorite collecting grounds at North Elba, New York. After 1914 he turned his attention more and more to the study of the flora of the Rocky Mountain region and the Pacific Coast States. Accompanied by his students, he conducted expeditions in Washington (1915), Tennessee and Kentucky (1916), Colorado (1917, 1920, 1928), Idaho, Wyoming, and Oregon (1922), Wyoming (1923), North Carolina and Tennessee (1924), and Upper Peninsula of Michigan (1927, 1929). These expeditions furnished him with an abundance of material critically studied in the field upon which his monographic studies and fungus floras were based. He described more than 200 new species of fungi. On these expeditions he collected not only fungi but also mosses, ferns, and flowering plants.

While his main interest was chiefly in the field of mycology, he contributed a number of papers on pathological subjects. The number of these, however, is by no means the measure of his interest in this field of research. He developed an outstanding course in forest pathology and stimulated his forestry students to keep alert to new problems in forest pathology. His interest in pathology is reflected in the problems of several of his graduate students who have published the results of investigations in this field. From 1917–1919 he was on leave from the University of Michigan, serving as pathological inspector on the Federal Horticultural Board.

In 1912 he was advanced to the rank of Assistant Professor and in 1920 to Associate Professor. In 1923 he was promoted to the rank of Professor of Botany.

In 1921 he was appointed Director of the University Herbarium, which, under his leadership, made rapid progress. The organization of the Herbarium as a strong research unit was one of his major achievements. His keen interest in the herbarium is indicated by the fact that he deposited there his personal herbarium, which consisted of thousands of specimens.

Throughout his life Doctor Kauffman retained his early interest in teaching. At Michigan he developed courses in algae, mosses, liverworts and ferns, and in mycology and forest pathology. His broad botanical interests are reflected in the diversity of the problems of his doctorate students. He not only had the ability to transmit his knowledge to his students but he inspired them to seek for information and to think for themselves. He always took an active interest in their problems and stimulated and encouraged them by his criticisms. His devotion to research, his boundless enthusiasm, and his unusual ability to detect the significant facts in research will be remembered as outstanding characteristics by all who came in contact with him.

Fortunately, a few weeks before his illness, his doctorate students expressed to him a little of their affection for him. At that time they pre-

sented a bas-relief of Doctor Kauffman to the University as a token of their appreciation of him as friend and teacher.

He was a member of The American Phytopathological Society, a fellow of the American Association for the Advancement of Science, and a member of the American Botanical Society, Torrey Botanical Society, Société Linnéène de Lyon, Washington Botanical Society, Michigan Academy of Science, Arts and Letters, Sigma Xi, and the American Forestry Association.

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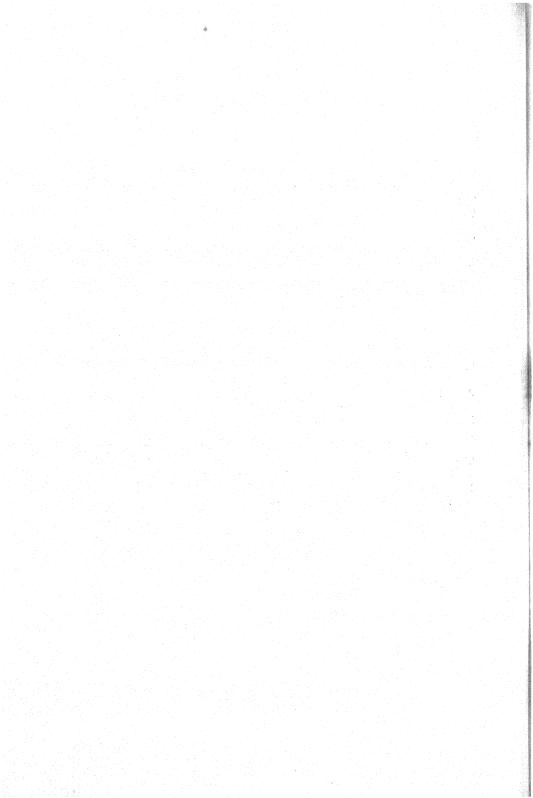
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EFFECTS OF ELECTROMAGNETIC WAVES ON FUNGI¹

FRANK H. JOHNSON

INTRODUCTION

In view of the increasing interest concerning the action of various rays on fungi the experiments described in this paper were undertaken with the idea of finding out to what portions of the electromagnetic spectrum three fungi, heretofore not studied from this point of view, were most susceptible to variations as a result of the rays. The species used were: Collubia dryophila Fr., Sclerotium bataticola Taub., and Fusarium batatatis Wr. Although previous investigators have, for the most part, studied in detail the effects of one particular type or sometimes two types of rays, the object of this study was, primarily, more to determine in a comprehensive way the reactions of the organisms to wave lengths representative of the major kinds of rays throughout as much of the electromagnetic spectrum as could be worked with in the laboratory, with certain limitations imposed by the difficulties of technique for working with some parts of the spectrum. The total range of wave lengths covered in this study extended from only a small fraction of an A.u. in the case of gamma rays to Hertzian waves of 50 and 100 m.

Inasmuch as a different technique was necessitated for experimentation with the different kinds of rays, only a few words concerning general methods need be mentioned here.

A sweet-potato-agar medium was used for pure cultures of the organisms. Except where stated otherwise, the pH of the medium was adjusted to 6.01 with a quinhydrone electrode. Inoculations, except in a few cases as described, were made by transferring a small bit of mycelium-bearing agar from a pure culture to the center of a Petri dish containing approximately 20 cc. of sterile medium. Irradiations were carried out as described under the separate headings.

REVIEW OF LITERATURE

a. Gamma and X-rays. The action of gamma and X-rays on fungi has not, apparently, been very extensively investigated, although considerable work has been done with Bacterium tumefaciens Smith & Towns. Actino-

¹ The writer wishes to acknowledge with much appreciation the assistance and generous cooperation, both in the use of apparatus and valuable suggestions for completing this work, rendered by the following: The staff of the Botany Department, N. C. State College of Agriculture and Engineering, particularly Dr. R. F. Poole and Dr. B. W. Wells; the Biology Department, Princeton University, especially Dr. Cary, Dr. Harvey, and Dr. Butler; the Physics Department, Princeton University, especially Professors Shenstone, Morris and Boice.

mycosis of the face and neck, caused by Actinomyces bovis Harz., has been successfully treated by a concentrated preparation of radium, filtered through 1 to 2 mm. of lead (10). Miescher found that prolonged exposure to radium rays retarded and finally suppressed development of cultures of Trichophyton gypseum and Achorion gypseum Bodin (14). Rivera (22) found that a capillary tube containing radium, suspended in test-tube cultures of Bact. tumefaciens, Bact. fluorescens (Flügge) Lehm. & Neum., and Penicillium crustaceum Fr., respectively, inhibited the growth of the organisms in the part directly exposed to the rays and concluded that gamma rays suppressed the growth and then beta rays killed the organisms.

Pichler and Wöber (18) tried the use of ultra-violet, X-rays, and radium for the control of bunt of wheat and smuts of barley, oats, and maize. Radium yielded only negative results, but successful treatment was obtained by ultra-violet and X-rays, especially in acid- and oxygen-containing solution, against Tilletia tritici (Bjerk.) Wint., Ustilago nuda (Jens.) Kell. & Sw., U. hordei (Pers.) Kell. & Sw., U. avenae (Pers.) Jens., and U. zeae (Beckm.) Ung. In X-ray experiments with T. tritici and T. levis Kühn, Rivera (21) obtained only negative results. Nadson and Philipov (15) found that sexual reproduction in pure cultures of young Mucor genevensis Lendn. and Zygorrhynchus Moelleri Vuill. was almost completely inhibited by 30 to 45 minutes of irradiation by X-rays, while weaker radiation stimulated development and induced mutations. The mutant characters were maintained in some cases through as many as 13 successive generations from spores of the mutants.

b. Ultra-violet rays. The portion of the spectrum usually thought of, and considered in these studies, as the ultra-violet region, extends from approximately λ 3900 to λ 1850.² The wave lengths below λ 1850, down to λ 1250, are generally designated Schumann rays. Ordinary atmosphere is practically opaque to wave lengths shorter than λ 1850 because of the high absorption of oxygen, and a special technique is necessary for studying the action of the waves on living material. Comparatively little is known concerning the physical properties of the waves between the very "soft," or long X-rays, which, above 10 A.u., have only a very short range in air, and the lower limits of the Schumann region into which the X-rays merge. The technique would be exceedingly difficult for studying the action of electromagnetic waves between 1 and 1250 A.u. on living organisms.

The first investigation of the effects of Schumann rays on protoplasm was made by Bovie (3), who studied the action of this light on a number of organisms, including rotifers, Spirogyra, amoeba, and spores of Penicillium, Cephalothecium roseum Cda., and Monilia sp. Spores were irradi-

² The Greek letter λ is used to denote wave length, here referring to Angström units.

ated in vacuo, while the other organisms were placed in a thin film of water on a flourite window of a hydrogen-discharge tube. The rays were found to have a lethal action, the time required for killing varying both with the species and individual organism, though, in general, a small organism was more easily killed than a large one. Sphaerella-like swarm spores were killed almost instantly. Bovie did not succeed in killing the tan-colored spores of P. brevicaule Sacc. or the black spores of Stemphylium sp. and concluded that it was because the Schumann rays did not have sufficient penetrating power to pass through the colored cell walls. By using a rocksalt screen, cutting off waves below \(\lambda\) 1800, it was demonstrated that the shorter wave lengths were the more destructive. A remarkable effect of the Schumann rays was their cytolytic action on cells. Bovie distinguished three types of photocytolysis in ciliated infusoria, which were quickly cytolyzed. Spores of Monilia, after exposure to the rays, when dry, and then allowed to absorb water until turgid, took on a coarsely granular, coagulated appearance.

Except for the Schumann region, more work has been done concerning the effects of ultra-violet light on fungi than any other portion of the spectrum, and it is under the influence of these rays that the organisms are, apparently, most susceptible to variation. By and large, the action of this light may be considered as of two kinds, which, to a certain extent, may take place concomitantly: it may be destructive, involving a suppression of growth or lethal effects on the mycelium or spores, or it may be stimulating to the formation of fruiting bodies. Lethal effects have been reported by a number of investigators. Fulton and Coblenz (9), working with a number of species of fungi, showed that ultra-violet radiations were destructive to spores after a comparatively short exposure on the surface of hard nutrient agar in Petri dishes. They found that the lethal dose varied with the species but that an interval of 45 to 60 seconds was generally quite efficient. They concluded that the fungicidal effectiveness of ultra-violet light depends on the intensity of the radiation and the wave lengths of the component rays. Lethal action was found to begin around 365 µ (undoubtedly µ µ was meant), as an upper limit, and to increase in effectiveness with decrease in wave length. Growing mycelium was found more sensitive than spores. Bovie (2) found that bacteria and spores of various fungi were killed by exposures of approximately 10 minutes to wave lengths shorter than λ 2925 but were not killed after 2-hour exposures to wave lengths above λ 3175. Tanner and Ryder (25) found yeast fungi quite susceptible to the lethal action of ultra-violet light, though, in general, less so than bacteria. The lethal dose varied with the species, ranging from 1.5 to 20 minutes. Pigmented yearts were found to be more resistant than those without pigment.

Their results indicated a direct relation between the size of the cell and its resistance to the light. Chyurlia (5) reported the alleviation of a case of bronchomycosis caused by Monilia Krusei Castel. under ultra-violet treatment, and Chavarria and Clark (4) have noted slightly inhibitory effects of ultra-violet light on nonpigmented pathogenic fungi of the skin. although the visible and near ultra-violet were stimulating in moderate doses. Lethal effects have been recorded by Sibilia (23) on the spores of Fusarium echinosporum Sib. and F. fuliginosporum Sib. Dufrénoy (7) observed cytolysis of the zoospores of Blepharospora irradiated for 2 to 3 minutes in a thin film of water. Lethal effects on the spores of Glomerella cingulata (Stonem.) S. and v. S. with a collapse and death of aerial mycelium in colonies 2 to 7 or 8 days old, were found by Stevens (24). Petri (16) tested the resistance of conidia of oak mildew to ultra-violet light and found that, although they germinated normally after exposures to wave lengths between λ 3900 and λ 4000, they were destroyed effectively by wave lengths from λ 2300 to λ 2500, with less effective action between λ 3130 and λ 3660. Acid media accentuated the destructive action. There was a high resistance to direct solar light.

In experiments conducted on a number of different fungi, Weston and Halnan (26) found in each case that the growth of the cultures exposed to the range of ultra-violet light passing through Sanalux or Vitaglass Petri dish covers from a quartz mercury-vapor lamp was suppressed. They noted that the mycelium was not killed but rendered "dormant" by the rays, and there was a tendency to grow deep into the medium to avoid the light. Porter and Bockstahler (19) found an inhibition of growth, together with a "collapse and probable death" of aerial hyphae in cultures exposed to the full range of ultra-violet light from a quartz mercury-vapor lamp. They noted, however, that with the exception of Sclerotinia sp., which was not killed by an irradiation of 30 minutes, their fungi were not actually killed after exposures of 60 minutes.

In addition to lethal or inhibitory action of ultra-violet light on the growth of fungi, the action on reproductive bodies is significant. Stimulation of the production of acervuli and perithecia in Glomerella cingulata, when exposed to the full range of ultra-violet light for as little as 2.5 to 4 seconds, has been reported by Stevens (24). The production of pyenidia in Coniothyrium was brought about earlier than normal by irradiations as short as 10 seconds. The effects of ultra-violet light on sporulation in Fusarium cepae Hanz. and Macrosporium tomato Cke. have been pretty thoroughly worked out by Ramsey and Bailey (20), who found that the most efficient wave lengths for increasing sporulation lay between λ 2535 and λ 2800. But they also found that abundant sporulation could be in-

duced by wave lengths above λ 3120 by exposure to direct sunlight and conclude that, if the intensity is great enough, wave lengths shorter than λ 3120 are not necessary for stimulation of spore production.

There does not seem to be any evidence that any of the variations quoted above have been due to a change in the culture medium as a result of the irradiations. There is evidence, however, that ultra-violet radiations produce changes in certain media that may affect the growth of the organism. Euler (8) obtained a better growth of *Penicillium glaucum* Lk. and *Rhizopus chinensis* Saito on media that had been irradiated for 2 minutes but a decreased growth on media that had been irradiated for 2 hours. Petri (17) found that the growth of *Blepharospora cambivora* Petri on carrot agar, sterilized and irradiated, was more vigorous. The same was true of *Deuterophoma tracheifila* Petri on boiled, irradiated milk.

c. Visible light, infra-red, and Hertzian waves. While there has been comparatively little investigation of the action of visible light and infra-red rays on fungi, it does not appear that such organisms are particularly sensitive to radiations in this region of the spectrum. Variations in the shape of colonies of Microsporon audonini Gruby and Achorion schoenleinii Leb. were found by Berde (1) to take place under various types of illumination. Porter and Bockstahler (19) found little marked differences in colonies of several fungi exposed continuously to various wave lengths in the visible spectrum, and to infra-red, though there was a tendency for cultures to grow less rapidly under any of the lights used than in darkness, especially so in cultures exposed to blue-green light. The most sensitive part of the colony to inhibition was the aerial hyphae. No change was observed in sporulation of Colletotrichum lindemuthianum (Sacc. & Magn.) Br. and Cay, under any of the lights, but a range of sensitivity to various wave lengths was noted in Cephalothecium roseum Cda. Cultures exposed through filters transmitting only infra-red rays underwent no modification. Cooper and Porter (6) found the vegetative growth of 2-day old cultures of Phytophthora paeoniae Cooper & Porter about the same when exposed for 4 days to various wave lengths and the full spectrum of visible light. No conidia or oospores were produced under red and yellow-orange filters, though they appeared abundantly under all others.

The biological effects of Hertzian rays is a comparatively new study. It is known that physiological changes in higher organisms may be brought about by the action of short Hertzian waves, but nothing has been recorded of their action on pure cultures of fungi. In week-old albino rats, death occurs, accompanied by a violent rush of blood to the fore and hind limbs and tail, when exposed to an electrostatic field of 100,000,000 cycles per second (13). The degree of external heat necessary to duplicate the effects

was found to be not less than 160° C., which is much higher than any measurable heat generated in the electrostatic field.

EXPERIMENTAL WORK

a. Gamma rays. The source of gamma rays used in the experiments was approximately 1 mgm. of radium bromide, sealed in a small glass tube. The alpha particles given off in the disintegration activity of the radium were undoubtedly stopped by the walls of the tube, and, while there was most probably a certain amount of beta radiation through the glass, it could not have been very intense because of the absorption in the glass and the small amount of radium.

The cultures in each case were exposed 4 at a time, by placing them vertically in a tin box with the radium between the 2nd and 3rd cultures. Four controls were shielded by lead, half an inch in thickness, from those being irradiated.

After continuous irradiation of Sclerotium bataticola for 72 hours, there was essentially no difference, either in total average areas attained by the mycelium, or the appearance of the cultures, between the irradiated and controls. The same was true with the acidity of the medium adjusted to a pH of 4.6 instead of 6.01. When, however, cellophane was substituted for the glass cover of the Petri dish immediately below the radium, there was a marked suppression of growth, manifested by a much thinner mycelium and delayed formation of sclerotia, which, after a period of 7 days, were far less numerous than in the other 3 irradiated cultures and controls. This effect was most probably due to the action of beta rays, whose intensity had been greatly reduced in the previous cases by the glass cover of the Petri dish but which penetrated the very thin cellophane in sufficient quantity to exert an effect on the organism.

With Fusarium batatatis, variations in pigment and sporulation occurred; but, after a number of control experiments, including several that indicated that the radium had no effect on the medium, they were apparently not the result of any action of the rays.

It was possible to use the half-culture method of exposure by cutting out plates of lead to shield $\frac{1}{2}$ of the Petri dishes, but no difference between the exposed and nonexposed portions of the cultures was observed after irradiations of 2 weeks' duration, from the time of inoculation, except where cellophane was substituted for the glass cover, as in the preceding organism. When this was done, the mycelium towards the radium was at first greatly retarded in development but later began to grow, with a large production of rhizomorphic strands (Fig. 1, A).

The half-culture method of exposure was used entirely with Collybia dryophila. The growth of this organism was suppressed in the part closest

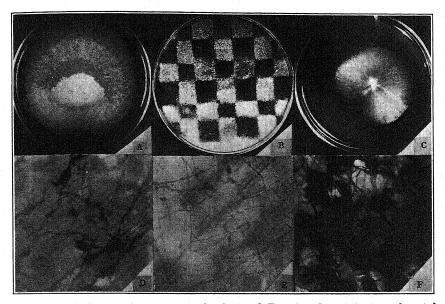


Fig. 1. A. Result of exposing \(\frac{1}{2} \) of culture of Fusarium batatatis to a glass tube containing radium, through cellophane cover, 9 days, beginning immediately after inoculation. Upper half shielded with a lead plate. The slight suppression of growth and increase in rhizomorphic strands in the lower half, probably a beta-ray effect. B. Culture of Sclerotium bataticola irradiated by ultra-violet light through a cellophane cover, 15 min. a day for 10 days, 8 in. from the lamp. Alternate areas of culture exposed to action of light by painting black squares on cellophane cover before first irradiation. Development of mycelium and sclerotia in exposed portions, represented by light squares; weak in comparison to those in shaded areas. (Photographed by transmitted light). C. Suppression of development of the mycelium of Collybia dryophila in part of culture nearest glass tube containing 1 mgm. of radium bromide. Probably a gamma-ray effect. D. Photomicrograph of the mycelium in situ in a culture of Sclerotium bataticola irradiated 20 min. daily for 4 days, 8 in. from ultra-violet lamp beginning 2 days after inoculation. E. Same as D. showing appearance of mycelium at surface of medium. F. Photograph of mycelium in same culture, taken about 2 cm. from that in D and E, showing normal development in portion of culture shielded from rays by a lead plate.

to the radium (Fig. 1, C). The inhibition took place only in the culture immediately above the radium, and, though it was slight, it must have been a gamma-ray effect. The failure to affect the culture below the radium could be accounted for by the slightly greater distance from the source of rays, whose intensity, of course, varies inversely as the square of the distance. The culture medium intervening between the radium and the organism that was inhibited would absorb a negligible amount of the very penetrating gamma rays.

With cellophane in place of the glass Petri dish cover, there was a complete inhibition of development. After 11 days the bit of mycelium used

to inoculate the culture had turned a brownish purple and was apparently dead, but, when removed from the radium, it soon began to develop and the new growth took on the appearances of the normal fungus.

b. X-rays.—Although about 500 cultures of the 3 species were experimented on, no modification was found that could be attributed to the action of X-rays. In some of the experiments on Sclerotium bataticola there was, apparently, a slight stimulation in the average rate of growth of irradiated over control cultures, but it was always so little as to be well within range of experimental error and the natural variability of the organism. In other experiments, under the same conditions, the average growth rate of the controls was slightly greater, so the net statistical results of a large number of cultures just about balanced. The half-culture method of exposure, with the aid of lead plates, was used as well as exposure of whole cultures with separate controls.

Except for a few preliminary experiments, cultures were all irradiated directly under the cathode. Table 1 gives a list of the conditions of exposure. An average of 16 cultures, with as many controls, was used in each experiment, and several experiments were repeated sufficiently to indicate that there was, most probably, no variation due to the X-ray treatment.

c. Schumann region of the ultra-violet.—In the present study a technique that takes advantage of the transparency of nitrogen to Schumann rays was used. The apparatus was constructed according to the following description.

To several 1-liter Pyrex Erlenmeyer flasks a short but wide glass tube that could be stuffed with cotton was sealed near the bottom. Short glass rods were sealed to the inside of each flask, about 1 cm. from the bottom and projecting horizontally from the sides, so that they could furnish a support for a pasteboard shelf, cut in a semicircle to extend halfway across the flask. Into the mouth of the flask was fitted a 4-hole rubber stopper, 2 holes occupied by heavy copper rods that extended down to within 31 inches of the bottom and the other 2 by glass tubes to allow an inflow and outflow of nitrogen. The inner ends of the copper rods were bent close together to serve as a spark gap. The glass tubes on the outside were bent in a double "S" to prevent spores and bacteria from drifting into the flask. for the inflow of nitrogen extended down to the pasteboard shelf on the inside, and the outer end was adapted to fit into a 2-hole rubber stopper to a wide-mouth bottle containing a mass of loose cotton. The flask and widemouth bottle with cotton were sterilized by hot air, while the rubber stoppers, with glass tubing and copper rods assembled, ready to be fitted into the flask and bottle, were wrapped in gauze and sterilized in the autoclave. A sterile culture medium was then poured into the flask to a depth of 1 to

TABLE 1.—Conditions of irradiation by X-rays of the 3 fungistudied, causing no variation in the organisms that could be attributed to the action of the rays

Organism	Tim of 1st	ne of ex hours 2nd	Time of exposure, in number of hours after inoculation st 2nd 3rd 4th 5th	in num 10culati 4th	on 5th	Length of exposure each time	Total length of ex- posure	Distance from cathode	Kilo- volts	Milli- amps.	Shortest wave length in Å. u.
Sclerotium		1				C		G	ē.		72 L 700
bataticola	н н	56 56				2 sees.	4 secs.	79 CIII	60 3	ne	117-105
	Н	26				;; 8 8	77 91	"))	3	22 22 23
	H	48				14 ''	28 ((÷ ;	ω,	23 23 23
	Нг	48 48 8				42 ··· 252 ···	84 '' 504 ''	;;;;	: :	: :	;; ;; ;;
	H	48			11,	10 mins.	20 mins.	77 77	,,,	"	" " "
	24	48	26	72	96	10 "	20 ,,	33 33	,,,	,	33 33 33
	24	48	26	72	96	15 ''	,, 92	"	;	ž,	22 22 23
	36					9 secs.	9 secs.	7, 97	120	20	.103
Fusarium											And the second s
batatatis	Н	48				2 secs.	4 secs.	79 cm	65	30	.207177
	Н	48				,, p	,, &	,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,	"	"	" "
	Н	48				; 80	16 ''	2,9 2,9	,,	, ,	" "
	72	104				10 mins.	20 mins.	31 (())	rc	" "
	72	104				50 "	40 (("	,,	,))))))
Collybia	109					40 mins	40 mins	2.7 cm	65	7.0	207-177
aryophila	127					-		i	}	,	

2 mm., the stoppers fitted into place, and the whole apparatus sterilized again in the autoclave. Several of such units were used in making the cultures to be studied. Inoculations were made through the tube near the bottom of the flask, after which it was closed with a rubber stopper.

As soon as the development of the mycelium was sufficient to allow one side of it to be well shielded by the pasteboard, irradiation was accomplished by means of a copper spark in an atmosphere of nitrogen. While it would not be possible to state the lowest limit of the waves that reached the organism, it is pretty certain that they extended well into the Schumann region. Nitrogen is transparent to Schumann rays, and, although the absorption increases regularly with decrease in wave length, it is very little as far down as $\lambda 1250$. (12). Data on water vapor are lacking, but there seems to be a maximum of absorption between $\lambda 1700$ and $\lambda 1600$, with a possibility of transparency on either side of this region. (12). The absorption of oxygen is very great for wave lengths below $\lambda 1850$, and, as it was impossible to get out quite all of the oxygen and the presence of water vapor was unavoidable, it is rather doubtful if any of the waves as short as $\lambda 1250$ reached the organism.

Nitrogen was passed into the flask for at least 10 minutes before irradiation was begun. In order to help remove the small amounts of oxygen present as an impurity in the nitrogen,³ it was bubbled through 3 bottles of pyrogallol connected in series with the tank of compressed nitrogen and the bottle of sterile cotton by glass tubing. A transformer was employed to step up the ordinary lighting current of 110 volts to about 12,000, which gave a spark producing a very intense ultra-violet spectrum. It was found that the heat from the spark was not sufficient to cause a rise in temperature of 2° C. in 5 minutes in the culture below it. When a total irradiation of more than 5 minutes was made it was made intermittently, so that heat effects would be sure to be avoided.

Because of the time-consuming nature of the experiments with the Schumann rays, it was not possible to study as many cultures as with the others. The results that were obtained, however, were consistent with those described by Bovie (3).

A three-day-old culture of *Sclerotium bataticola* was irradiated for a total of 20 minutes. When a microscopic examination of the mycelium was made 4 hours later, striking differences were revealed between the control and irradiated sides of the culture. The protoplasm in the cells of the mycelium that had been irradiated was discontinuous, had a coagulated appearance, and was much more granular than the control. Towards the periphery the mycelium was composed chiefly of long, straight hyphae, with

³ This method is not, of course, very efficacious for purifying nitrogen, but apparatus for burning out the oxygen was lacking. Small amounts of oxygen that reached the flask were probably consumed at once by the copper spark.

very few side branches; while, in the shielded area, the side branches were numerous, which would seem to indicate that during the 4 hours intervening between the experiment and the examination, little or no growth had taken place in the irradiated portion. Subsequent experiments showed that it was very difficult to kill a whole area of mycelium by these rays, probably because they are so easily absorbed that they do not penetrate deeper than the uppermost cells. Older mycelium was found much less susceptible to the injurious action of the rays, but essentially the same phenomena occurred to a less degree after exposures of 20 minutes on cultures over a week old. There were always a collapse and withering of the aerial mycelium, effects that occurred to some extent in short exposures of 4 or 5 minutes.

Since Fusarium batatatis does not grow so fast as Sclerotium bataticola, 5-day-old cultures were the youngest that were irradiated. Ten minutes was found sufficient to cause a collapse of the aerial mycelium and an increase in pigment that became apparent within 24 hours. The mycelium was not entirely killed, however, by exposures of 25 minutes, for new growth would take place within 2 days out from below the superficial layer. Microscopic examination of the superficial portion of the irradiated mycelium revealed a very granular protoplasm, and the granules were relatively large. The most striking effect was the formation of numerous bulbous enlargements at various points along the cells of the mycelium. Sporulation of both micro- and macrospores was abundant in both irradiated and nonirradiated portions but considerably more abundant in the former. A number of examples could be found in the irradiated area of spores on the ends of hyphae. In many cases there was a partial constriction, as if they had started to break off and had been killed or as if sporulation had been induced and, in these cases, was incomplete.

Eight-day-old cultures were found less susceptible to injury than those only 5 days old; but, as in the preceding species, essentially the same phenomena occurred, to a less degree, after exposures of 15 to 25 minutes.

Collybia dryophila grows more slowly than Fusarium batatatis; so, it was necessary to wait a week after inoculation before the mycelium had developed to an extent that allowed good shielding of half of it for a control. An irradiation of 20 minutes on 7-day-old cultures arrested the growth on one side, for a period of about 3 days, after which new mycelium would develop out from under that which had been irradiated. Two exposures of 20 minutes each, a week apart, sufficed to kill the mycelium, a fact proved by inoculating new cultures from it. Normal development proceeded in all new cultures inoculated from the shielded side, but no growth took place in those inoculated from mycelium that had been irradiated a total of 40 minutes. Twenty-minute irradiations caused the organism to pigment with a brownish color, separated by a sharp line from the control

side. The surface of the irradiated side took on a smooth, powdery appearance, probably due to the collapse of aerial hyphae. The most striking macroscopic differences were those in size and color of the 2 portions of the culture. Microscopic examination after a 20-minute exposure revealed phenomena similar to the preceding cases in that there were a coagulation of the protoplasm of the overlying cells and the formation of rather large granules.

By using $\frac{1}{2}$ of the same culture as a control in the manner just described, there was a maximum uniformity of conditions, with the light as the only difference; so, there can be little doubt that the results were brought about by the action of the rays, alone.

d. Ultra-violet rays.—The source of the ultra-violet light used in the following experiments was a Hanovia Universal Laboratory Model Ultra-Violet Lamp, operated on direct current, 110 volts and 5 ampères. An electric fan was placed where it could constantly ventilate the quartz tube of the lamp. By this means overheating of the tube was avoided and an additional advantage was secured, for, with the tube kept at a constant temperature, the ampèrage through the lamp was found not to vary so much as 0.2 of an ampère during long exposures of over an hour. Irradiations were not begun until the lamp had been in operation a few minutes, in order to secure uniform intensity of illumination. To get the most nearly vertical rays irradiations were never made on more than 4 cultures at a time. Inasmuch as the half-culture method of exposure was used almost entirely, the parts of the organisms to be irradiated could always be exposed directly under the quartz tube. The part of the Petri dishes on the side away from the tube was covered by a lead plate.

In order to utilize the shortest wave lengths given off by the lamp and at the same time maintain the purity of the cultures, a new technique of irradiation was devised, embodying the substitution of cellophane in place of the glass cover of the Petri dish (11). The spectrograms (Fig. 2) of the lamp used in the experiments give the transmission of the cellophane as well as the filters that were used to cut off the shorter wave lengths. Cellophane transmits the full range of the light given off by the lamp with practically no absorption apparent from the photographs of the spectrum. Spectrograms taken on a spectroscope focussed for the short wave lengths showed that the lowest limit of the waves given off by the lamp was actually around $\lambda 2000$. Photographs of a copper spark, on the same spectroscope, taken through the various filters, showed that the actual limit of transparency extended to lower wave lengths than are registered in figure 2 from the mercury-vapor lamp. The practical limit of transmission in each case may be considered as follows:

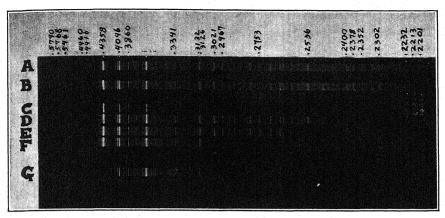


Fig. 2. The mercury-vapor spectrum of the lamp used in the experiments, with the transmission of the different filters. (Some of the lines were lost in the printing). A. Mercury-vapor spectrum, no filter, λ5790 to λ2201. B. One thickness of cellophane, λ5790 to λ2201. C. Culture medium, 2.5 mm. in thickness, λ5790 to λ2536. D. Filter I (Corning 980 A), λ5790 to λ2302. E. Filter II (Vitaglass), λ5790 to λ2536. F. Filter III (Corning G 86 B), λ5790 to λ2907. G. Filter IV (Corning G 585 A), λ4096 to λ3126.

Filter I (Corning 980 A) λ2302; filter II (Vitaglass) λ2536; filter III (Corning G86 B) λ2907; filter IV (Corning G 585 A) λ4096 to λ3126.

To test out the sensitivity of each species to the lethal action of the rays 3 experiments were done in a similar manner. Into each of 25 test tubes, 5 cc. of distilled water and 10 lead shots were put. The test tubes were plugged with cotton and autoclaved. When cool a small piece of mycelium bearing agar was introduced from a pure culture into each test tube. By shaking thoroughly, the mycelium was broken up, and, in the case of Fusarium batatatis, the spores disentangled from it. The liquid in the test tubes was then poured over the surface of stiff agar culture medium in Petri dishes, cellophane covers applied, and the newly inoculated cultures irradiated at once for periods ranging from 1 to 50 minutes, 8 in. from the lamp. Five cultures were reserved as controls. With each species, there was hardly any difference in the subsequent development of exposed and unexposed cultures. All germinated normally, and there was abundant germination from spores of F. batatatis, even in the cultures irradiated 50 minutes. The spores were not covered by as much as 1 mm. of water. The discrepancy between this and the results of other investigators, who have recorded lethal effects on spores following irradiations for intervals as short as 1 minute (9), seems rather too large to be accounted for only by the differences in sensitivity of different species. The same results were obtained in the present experiments when the cultures were exposed to the direct action of the rays without the cellophane covers. The intensity of the light is, no doubt, an important factor and may account to some extent for the rather large discrepancies. Accurate comparisons cannot be made between the work of different authors without a record of the intensity, as nearly exactly as possible, of the light used in their experiments. Furthermore, it might be well to point out here that comparisons of the action of different wave lengths, where glass filters are used to eliminate certain portions of the spectrum, are of little significance without a knowledge of the percentage transmission of each filter, and a regulation of the amount of the light reaching the organism, so as to be equal. For a comparative study of several species, however, when the same filters are used in all the experiments, the results are qualitatively perfectly good.

For the remainder of the experiments the half-culture method of exposure was used entirely, except in one case (Fig. 1, B), and, mostly, according to the following scheme of varying the intensity of the light, by distance and length of exposure, as follows:

8 inches, 5 minutes; 8 inches, 10 minutes; 16 inches, 5 minutes; 16 inches, 10 minutes.

Cultures were irradiated after the mycelium had grown to an area of about 2 cm. in diameter. This was attained in *Sclerotium bataticola* from 36 to 48 hours after inoculation; in *Fusarium batatatis*, 3 to 4 days; and in *Collybia dryophila*, 5 to 6 days. Irradiations were generally made every 24 hours for a period of 5 days.

Under cellophane, alone, 5 minutes' irradiation a day, at 16 in., was sufficient to cause a noticeable suppression of growth in Sclerotium bataticola, with an increase in pigment in the mycelium and a tendency for it to grow deeper into the medium. These reactions occurred to a greater extent with longer exposures at less distance from the lamp. (Fig. 1, D, E, and F.) There was an inhibition of the formation of sclerotia, along with the increase in pigment, but sclerotia would continue to form slowly in parts of cultures irradiated daily for 15 minutes, at 8 in. Figure 1, B, shows very clearly how the action of the light inhibited sclerotial formation, and, although it is not apparent in the photograph, this culture demonstrated particularly well the manner in which the mycelium grew deeper into the agar under the influence of the rays. In the black squares, which represent the shaded portions of the culture, the mycelium continued to develop until these areas were over 2 mm. higher than the light squares, which represent the irradiated areas, in which there was scarcely any mycelium on the surface of the medium.

4 Control experiments, in which half-portions of the medium in Petri dishes were irradiated before inoculation, indicated that the light did not bring about reactions in the medium that might affect the organisms.

Under filters I and II, cutting off wave lengths below $\lambda 2302$ and $\lambda 2536$, respectively, there was very little difference between the two sides of cultures irradiated daily for 10 minutes, at 8 in. There was a slight suppression of the aerial mycelium, and an increase in pigment that was never very striking. The variations were usually most noticeable after the third exposure and to a greater extent under filter I than filter II but were generally blotted out, so to speak, by the rapidly growing mycelium after the fifth exposure, when the cultures were a week old. This effect would seem to indicate that the young mycelium is more sensitive to the rays than the older, a fact which might account, to some extent at least, for the apparently great resistance to the 50-minute exposures in the first experiment.

There was no effect under filter III, which cuts off the rays below $\lambda 2907$, of filter IV, transmitting between $\lambda 4096$ and $\lambda 3126$, after irradiations as long as 2 hours and 15 minutes at a distance of 8 in.

With Fusarium batatatis,5 as with the preceding species, the most pronounced reactions occurred under cellophane, alone. Under filters I and II. the same types of reactions occurred, to a less degree, as under cellophane. The effects of the light, under these filters, were much more prominent with this species than with Sclerotium bataticola. Daily exposures of 5 minutes, at 8 in., caused a very pronounced increase in pigment and a very noticeable suppression of growth. (Fig. 3, A and B). Semicircular rings appeared in the mycelium at points corresponding to the extent of its development at the time of each exposure. This was an interesting phenomenon because it occurred only under certain conditions in each of the 3 organisms studied. It was correlated with at least two things: (1) the natural growth rate of the organism and (2) the filter under which it was irradiated. With F. batatatis they were most prominent under filter I but were noticeable under all of them whenever the length of exposure was sufficient to produce a reaction. With S. bataticola they occurred only under cellophane and with exposures of between 5 and 20 minutes daily at 8 in, or longer exposures at 16 in. There never appeared more than 3 rings in this species because they always appeared at a point corresponding with the extent of the mycelium at the time of irradiation, probably because the youngest mycelium was the most sensitive to the action of the rays, and within 3 days after the first irradiation the mycelium of this organism had covered the surface of the medium in the Petri dish. With Collybia dryophila the rings did not form at all under cellophane but occurred under both filters I and II whenever the exposure was long enough to produce any inhibition of growth. An explanation for this phenomenon will be suggested farther on in the discussion.

Under filter II, the only reactions that occurred in Fusarium batatatis after weak exposures, such as 5 minutes a day, at 16 in., were a very slight

⁵ Differential counts of sporulation were not made in this study.

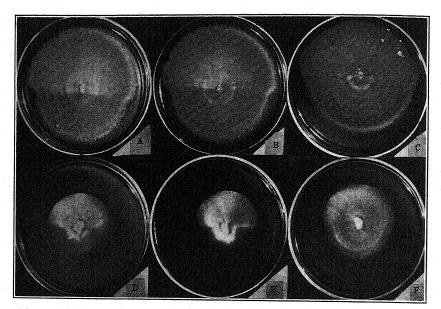


Fig. 3. A. Fusarium batatatis, after exposing lower half of culture to full range of ultra-violet light through cellophane cover, 10 min. a day, for 5 days, at 8 in. from the lamp, beginning 3 days after inoculation. B. Same as A, except that exposures were only 5 min. a day. C. Same as A, but with filter I, cutting out rays below $\lambda 2302$, covering exposed part. D. Collybia dryophila, after exposing lower half to full range of ultra-violet light through cellophane cover, 10 min. a day, for 5 days, 8 in. from lamp, beginning 5 days after inoculation. E. Same as D, except exposures were for only 5 min. a day. F. Same as D, but with filter I, absorbing rays below $\lambda 2302$, covering part of culture exposed to rays.

increase in pigment and very faint formation of rings. The reactions under filter I were always greater for similar conditions of exposure than under filter II. Under filters III and IV no variation occurred after exposures as long as 3 hours at 8 in.

With Collybia dryophila there was a pronounced suppression of growth after exposures of 5 minutes daily under cellophane at a distance of 8 in. (Fig. 3, D and E). There was no increase in pigment, although it will be recalled that such an effect occurred in the experiments with Schumann rays. The inhibition in growth was not so great as the photographs might indicate, for, when the cultures were held up to the light, it could be clearly seen where development of the mycelium had taken place under the surface of the medium. Under filters I and II, especially the latter, the inhibition was much less striking (Fig. 3, F). It is interesting to note in this connection that the 2- to 3-mm. thickness of culture medium has approximately the same lower limit of transmission of the rays as filter II, so the development of the mycelium deep in the medium is readily understandable. When the

daily exposures were discontinued and the culture set aside the underlying mycelium would soon return to the surface and continue to develop normally at a point about 2 mm. beyond where it was, below the surface, at the time of the last exposure.

As with the preceding species, there was apparently no effect at all under filters III and IV, after exposures as long as 3 hours at 8 in.

Returning to the phenomenon of ring formation under the action of the light, it has already been stated that this was correlated with the filter used and the natural growth rate of the organism. From the observations made in the present study the mechanism for this phenomenon, which is no doubt partly in adaptation to injurious action of the rays, depends on the alternate development of the mycelium deeper in and, then again on, the surface of the medium. This depends, first, on whether the action of the rays is strong enough to cause the mycelium to grow into the medium, in spite of other adaptive responses, such as pigmentation, which may take place in resistance to the light. The wave lengths transmitted by cellophane were obviously those that produced the greatest effect, while filters I and II, successively, absorbed more of the wave lengths capable of bringing about ready response in the organisms. Bearing these facts in mind, together with the fact that when rings appeared, at all, they appeared at points corresponding with the extent of the mycelium at the time of the last irradiation, an explanation for the formation of the rings in the cultures as described above suggests itself at once. They appeared in Sclerotium bataticola only under cellophane, alone, because the other filters absorbed the waves capable of causing the mycelium to grow deeper into the medium. This organism grows so fast that, in the 24-hour interval between exposures, the mycelium returned to the surface where development continued rapidly until the next irradiation, which slowed it up and sent it down into the medium again. Collybia dryophila showed no signs of rings under cellophane, alone, because it grows so slowly that after an initial irradiation sufficient to cause it to grow into the medium, there was not enough time in the 24-hour interval between irradiations for it to return to the surface and undergo any considerable development there. Rings appeared in this organism under filters I and II because these filters cut out the more effective rays and caused only a partial growing of the organism into the medium. The mycelium was not affected to the extent that it could not continue developing well on the surface. Fusarium batatatis, which has a growth rate about midway between those of the other two fungi, showed the formation of growth under cellophane and filters I and II because the rays transmitted by each had an effect sufficient to cause the mycelium to grow into the medium, while the growth rate was fast enough to allow new development on the surface during the interval between exposures. The rings were most prominent under filter I because the wave lengths transmitted by cellophane exerted an effect great enough partially to blot them out, so to speak, while the wave lengths transmitted by filter II did not exert an effect sufficiently great for them to become very prominent. While this hypothesis for the cause of ring formations, as described, is evidently true, from observation of a large number of cultures, it is not offered as the whole story underlying the formation of rings in the present study nor as an explanation for the occurrence of such structures in pure cultures of fungous organisms in general. Factors other than those of natural growth rate of the organism combined with the action of different ranges of wave lengths of the light no doubt enter into the cause of the phenomena, but the correlations described are perhaps interesting for whatever they may be worth.

One thing was very consistently observed in the experiments with these rays, viz, that there was often very little difference in the 5- and 10-minute exposures on an organism at a given distance. It would be practically impossible to obtain accurate numerical data in support of this statement, but it is almost certain that in no case did a 10-minute exposure produce twice the reaction of that of a 5-minute exposure, at the same distance, for it was often hard to tell them apart from the appearance of the cultures. Likewise, doubling the distance did not seem to reduce the effect by anything like as much as $\frac{1}{4}$ for exposures of corresponding lengths of time, although the intensity of the light was reduced by that much.

To determine the relative sensitivity of Fusarium batatatis and Collybia dryophila to the light transmitted by the different filters, a uniform procedure of irradiation was arbitrarily chosen. The length of the exposure, at 8 in. from the lamp, which was just sufficient to cause a variation in the organism within 24 hours after irradiation, was determined. Irradiations were made when the mycelium had developed to an area of about 2 cm. in diameter. After a few preliminary experiments to find the approximate length of exposure needed, a series of cultures of each organism was irradiated for gradually increasing lengths of time in order to get more nearly exact data. While there was some variability in the lengths of exposures for individual cultures, the results, after repeated experiments, were, on the whole, fairly consistent. The lengths of exposures varied within the limits set forth below:

Filter	$F.\ batatatis$		C. dry	ophila
Cellophane alone	10 to 15 seconds	$1-2\frac{2}{3}$	to 21	minutes
Filter I	3½ to 4 minutes	$5\frac{1}{2}$ to	6	"
Filter II	4 to 5	61 to	63	"

e. Visible light rays. The apparatus for irradiating with visible light rays consisted of a large wooden box, painted black inside and out, that

could be opened from the side in order to put in or remove cultures to be exposed. It was divided into 3 light-proof compartments, with 2 holes, each 4 in. square, cut in the top of each. These openings could be covered by filters transmitting different ranges of wave lengths. Above each hole was a 150-watt globe that was lowered into a glass battery jar supplied with a constant stream of cool water. The water spilled over the tops of the iars into Pyrex pans that were slightly tilted and sealed to a drain by means of beeswax and paraffin. The water kept the globes perfectly cool and absorbed the infra-red rays. The temperature inside the box was seldom found to vary more than 2 degrees on either side of 22° C. The intensity of the light was reduced by having to pass through the water. the glass of the battery jar and Pyrex pan, and the color filter before reaching the organism on the floor of the box 21 feet below the filament of the globe, but these precautions were found necessary in order to maintain a constant temperature favorable for the growth of the cultures and avoid possible heat effects.

In addition to the full range of visible light, violet, blue, orange, and red color filters were used. None of the filters was quite spectrally pure. The half-culture method of exposure was used entirely, by means of pasting a strip of black paper over half of the top of the Petri dishes.

No positive or consistent variation was noted in irradiated cultures except in one case. When Fusarium batatatis was exposed to the full range of visible light for a period of a week or more, starting with the time of inoculation, there was a slight but distinct increase in pigment. Sporulation was abundant under all filters. There was no evidence of any influence on the growth of the mycelium in any of the organisms, after exposures of over 2 weeks beginning right after inoculation. These particular organisms may be naturally more resistant to the action of visible light rays, or perhaps the intensity of the light was not so great as that which caused variations in other organisms described by previous investigators (1), (6), (19).

f. Infra-red rays. The source of the infra-red radiations was a 150-watt globe, placed where it could be constantly ventilated by cool air through an open window, $2\frac{1}{2}$ ft. above the sill. Cultures of the organisms were placed on the sill in a flat pasteboard box, with an opening 4 in. square, cut in the top. The opening was covered by a Wratten and Wainwright stained gelatine light filter, transmitting no waves below $\lambda 6900$ and less than 5 per cent below $\lambda 7000$. One-half of each of 2 cultures could be exposed at the time under this filter.

The results were entirely negative. There was apparently no effect on any of the organisms after exposures lasting as long as 10 days, starting immediately after inoculation.

g. Hertzian waves. The half-culture method of exposure was possible with Hertzian waves by means of wrapping half of the Petri dish with

lead foil to neutralize the field in that portion and exposing the other half between a condenser made of 2 copper plates, 4 in. square. The electrostatic field between the plates oscillated with a frequency that gave a wave length of 100 m. in the first experiment and 50 m. in the second. A UX 210 tube was used in the set.

The results of the experiments with Hertzian waves that have been completed so far have been entirely negative. There was no effect on 3- and 4-day cultures of Fusarium batatatis, on 4- to 8-day cultures of Collybia dryophila, or 3- to 4-day-old cultures of Sclerotium bataticola as a result of constant irradiation for 65 hours by a wave length of 100 m. and for 48 hours by a wave length of 50 m.

DISCUSSION

The present study, covering as it does such a broad range of wave lengths through the electromagnetic spectrum, on 3 different fungi, is by no means an exhaustive one. It seems sufficient to indicate, however, that the action of rays in certain portions of the spectrum are more likely to cause variations than in others. The fungi studied were all sensitive to more or less the same ranges of wave lengths, and the variations that occurred differed more in degree than in kind.

The results were necessarily inconsistent with those of some of the previous investigators, for the review of the literature, especially in the case of the ultra-violet rays, revealed a good many apparently wide discrepancies between the results of different workers (18), (21), (15), (9), (2), (16), (19), (1). On the whole, the discrepancies were more quantitative than qualitative, which is perhaps as much the result of different conditions of experimentation as of differences in the organisms. The rôle of the intensity of the various rays has been little and unsatisfactorily touched upon so far; and, although it is a complicated study, requiring careful and sometimes tedious as well as difficult calibration of instruments in order to secure accurate data, it might yield valuable results. Until such a procedure is adopted, accurate quantitative comparisons between the work of different authors, as well as between the action of different wave lengths, are impossible.

The only rays, in the present study, that were effective in causing variations in the organisms were those in the Schumann and short ultraviolet regions, from $\lambda 1250$ up to $\lambda 2536$, although variations might have occurred under the influence of other rays had the intensity been great enough. No effect occurred as the result of X-rays, and only a very slight effect, evidently due to gamma rays, occurred with radium. Inhibition in growth of all 3 fungi occurred with radium, but, except for a slight inhibition in the case of $Collybia\ dryophila$, the effects were probably caused by

heta rays instead of the electromagnetic gamma rays. Ultra-violet rays caused a suppression of growth, together with reactions that may be considered as adaptive against the injurious actions of the light, while the Schumann rays apparently exerted such destructive effects on those cells of the mycelium to which they penetrated that the organisms could not undergo adaptive responses in resistance to their action. The problem of adaptive responses is an interesting one, and it is here suggested that such responses, manifested under the influence of the ultra-violet light in such ways as the increase in pigment and growing of the mycelium deeper into the medium, may be responsible for the evident failure of the organisms to react in direct proportion to the length of the exposure or inversely to the square of the distance from the source of the rays. It is, in the first place, necessary to bear in mind that in working with living organisms a whole series of complicated reactions may come in to upset, to a certain extent, the exact conformity to physical laws that hold for less complex hodies. Assuming that the action of the short ultra-violet rays is of an injurious nature, as it evidently is, the natural adaptability of the organism to the light is suggested as one of the factors that may throw the variation slightly out of proportion with the intensity of illumination and length of exposure. The adaptability of the organism is little more than a name for a number of reactions that may take place entirely unobserved within the organism or become evident through variations in pigment and the methods of growing into the medium. Under a given set of conditions there is probably a definite threshold for the amount of light necessary to make a variation become evident in the organism. The threshold of a given organism would undoubtedly vary under different conditions, depending, for example, on whether the irradiation was by light of great or less intensity and on the age of the culture. If there were a very accurate way of securing numerical data on this subject, the thresholds would be a good way of determining the relative sensitivity of different organisms to various wave lengths. In working out the minimal lengths of exposure necessary to cause a variation within 24 hours, under a uniform set of conditions, for Fusarium batatatis and Collybia dryophila, the results were consistent enough to show clearly that a minimum amount of light is necessary for a variation to become evident, and above this the variations occurred comparatively rapidly. It is interesting to note that the threshold for C. dryophila, which produces a white mycelium, was higher in each case than that for F. batatatis, which is pigmented and may become heavily so under the influence of the rays.

The fundamental nature of the reaction of the organisms to various wave lengths can hardly be conjectured at the present stage of biophysical knowledge. Wave length is evidently of great significance, and apparently

a small intensity of some wave lengths exerts a considerable effect, while other wave lengths might cause no reaction, even though of great intensity.

SUMMARY

- 1. A study of a total of over 1,500 cultures of 3 fungi was made under the influence of gamma rays, X-rays, Schumann rays, ultra-violet rays, visible light rays, infra-red rays, and Hertzian waves.
- 2. Collybia dryophila, Fusarium batatatis and Sclerotium bataticola were found more susceptible to variation under the influence of the rays in some portions of the spectrum than in others.
- 3. A slight inhibition of growth in the mycelium of *C. dryophila* occurred in the parts of cultures closest to the tube of radium. This was evidently a gamma-ray effect. The inhibition of growth in cultures of all 3 organisms, which occurred to the greatest extent in *C. dryophila*, whose development was completely arrested, when exposed to the radium through cellophane covers, was probably a beta-ray effect and hence not due to electromagnetic waves.
 - 4. No variation was observed under the influence of X-rays.
- 5. Schumann rays were the most destructive but did not affect so large a proportion of the mycelium as the ultra-violet rays proper, probably because the former are so easily absorbed that they did not penetrate deeper than the uppermost cells. In all 3 organisms, Schumann rays caused an apparent coagulation and granulation of the protoplasm in the overlying cells of the mycelium.
- 6. Ultra-violet rays had a greater or less effect, depending on whether irradiations were made through cellophane, which transmits the full range of the spectrum from a quartz mercury-vapor lamp, or filters that cut off the shorter wave lengths. The rays passing through Corning filter 980 A. cutting off wave lengths below \(\lambda 2302\), had less effect than those through cellophane, which extended down to around \2000, but exerted a greater effect than the rays passing through vitaglass, which transmitted down to λ2536. An increase in pigment, together with a suppression of growth, occurred in F. batatatis and S. bataticola. Collybia dryophila underwent a suppression of growth but no increase in pigment. The formation of rings in the mycelium of the organisms, under the influence of the light, was correlated with the natural growth rate of the organism, and the wave length and intensity of the light. The responses of the organisms did not seem in strict proportion to the length of the exposure or intensity of the light, possibly because adaptive reactions of the organisms may slightly displace the physical laws holding for less complex bodies. Under an arbitrary set of conditions, a somewhat variable 'threshold' was found, up to which no effect of the radiations could be observed in F. batatatis and C. dryophila.

- 7. The only effect of various wave lengths and the whole range of visible light was an increase in pigment in F. batatatis after exposures of a week or more to the full range.
 - 8. No effect was found as the result of infra-red rays above $\lambda 7000$.
- 9. No effect was produced in the organisms by Hertzian waves of 50 and 100 m., respectively.

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TRANSMISSION OF THE PINEAPPLE YELLOW-SPOT VIRUS BY THRIPS TABACT¹

MAURICE B LINEORD

Pineapple yellow spot is a virosis that has risen to prominence recently in the Hawaiian Islands, where it is one of the major diseases of that unusual plant, Ananas sativus L. The writer, in a preliminary note (5) has set forth his findings that the most important vector, if not the only one, operating in the field occurrence of this disease, is the onion thrips, Thrips tabaci Lindeman. It is the purpose of the present paper to report in more detail the studies upon which that note was based and to present the findings of further studies dealing with the transmission of the yellow-spot virus. The methods of rearing and handling the experimental insects are described in detail because work with thrips presents some special problems not involved with larger insects and because the technique used in this work is quite different from that employed by Samuel, Bald, and Pittman (7) in their work with Frankliniella insularis. Other aspects of the disease will be considered in other papers.

FORMER INVESTIGATIONS

Investigations, instituted by the staff of the Experiment Station of the Association of Hawaiian Pineapple Canners shortly after the disease was first seen in 1926, has been in progress continually up to the time the writer assumed his present duties in July, 1929. Although the means of transmission was not then known, much had been learned about the disease that aided subsequent work. A preliminary description of the disease and a partial statement of this earlier work have been prepared by Illingworth (2), formerly Entomologist at this Station. Figures 1 and 2 illustrate the outstanding symptoms.

Before the writer took up this work, the view had already been developed by G. E. Paxton² that yellow spot was a virosis. He based this opinion upon his studies of symptoms, stressing the chlorotic rather than the necrotic aspects of the disease and upon the absence of demonstrable microorganisms from the chlorotic tissues. Mr. Paxton, after failing to transmit the disease by various methods of mechanical transfer of the juices of diseased plants, had postulated, furthermore, that this virus required a specific insect vector for its transmission. The fact that the first symptom of this disease is an *initial spot*, which develops characteristically

¹ Technical paper No. 24 of the Experiment Station of the Association of Hawaiian Pineapple Canners, University of Hawaii.

² Mr. Paxton reported these findings in the private organ of the pineapple industry.

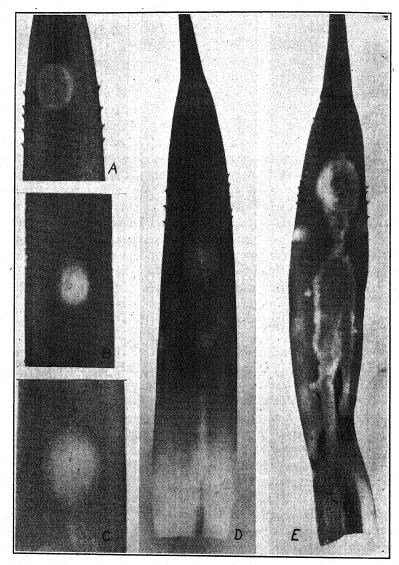


Fig. 1. Initial and later symptoms of yellow spot in pineapple. A, B, and C. Representative initial spots or chlorotic local lesions. D. Somewhat later stage, showing a characteristic initial spot with a yellow streak below it ending in a water-soaked streak at the base of the leaf. E. Later stage of an initial-spot leaf, showing marked necrosis.

a few inches from the base of an elongating leaf, suggested to him that these spots were the points at which the leaf had been inoculated and that the vector was able to inoculate only at or near the base of a growing leaf.

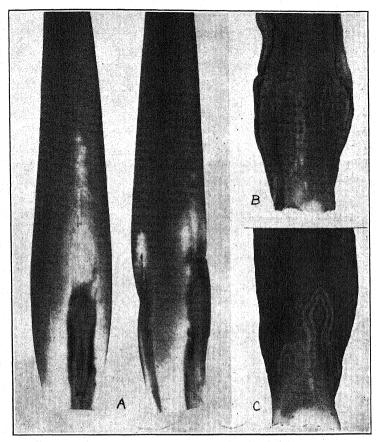


Fig. 2. Pineapple yellow spot in leaves from above the initial-spot leaf. A. Two leaves from a field specimen, showing chlorosis and the necrosis that characteristically follow. B. and C. Unusually elaborate development of chlorotic ring and line patterns on leaves of a seedling plant from the greenhouse.

Elongation of the leaf from its base during the incubation period of the virus in the leaf he held to account for the appearance of the new initial spot several inches up the leaf.

PRELIMINARY STUDIES

Search for the vector. A critical review of the evidence with Mr. Paxton led the writer to accept the above hypotheses as a working basis and to center attention upon the initial spots. Microscopic examination of such spots by R. N. Chapman in the hope of discovering clues as to the nature of the insect that might have fed there revealed the presence of characteristic and minute punctures in the upper surface of the leaf near the initial

spots. Further studies by the writer showed these punctures (Fig. 3, a), which were taken to be feeding punctures made by a relatively large insect, to be almost constantly associated with initial spots. They do not always occur within the spot itself and generally there are none at the center of the spot. They are, however, much more abundant near an initial spot than on other parts of the leaf and are not commonly present on leaves of healthy plants. Subsequent examinations of numerous collections from different islands and at different times of the year have shown these punctures to be associated with nearly every initial spot of the disease. In appearance they have been so uniform throughout as to indicate that they are the work of either a single species or a group of closely related species of insects.

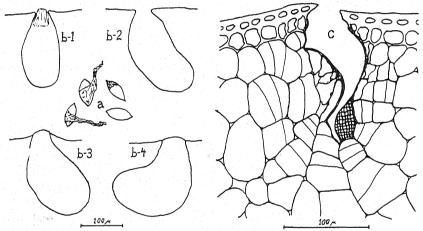


Fig. 3. Thrips ovipunctures associated with initial spots on pineapple leaf. a. Empty ovipunctures as seen from the leaf surface. Note the shrivelled prong that stands up from some of the orifices. This is pulled out by the emerging larva. b-1 to b-4. Relatively fresh ovipunctures from which the larvae have already emerged, as seen in cross-sections of the leaf. Observe that b-1 and b-2 may owe their different shapes to different angles of sectioning. c. An old ovipuncture that is nearly filled up by growth of the surrounding leaf tissue. All drawn with camera lucida. Note that c is enlarged more than a and b.

Microscopic study of sections of the leaf cut through such punctures at different angles (Fig. 3, b) showed that what had been seen from the surface was only the narrow orifice leading to a reniform cavity within the leaf. The shapes of different individual cavities and the conditions of the surrounding cells of the pineapple leaf (cf. Figs. 3, b, and 3, c) indicated that an object had filled the cavity for a time and had then escaped. These studies showed the typical size of the pouch to be about $100 \times 200~\mu$, as seen in longitudinal section. The finding of egg membranes in some sec-

tions completed the evidence that these cavities were the empty egg-punctures of a very small insect.

Other inconspicuous markings on the leaf surface were then recognized to be probably the feeding marks of the insect that had made the larger punctures with its ovipositor. This feeding injury, like the ovipunctures, was found much more commonly on initial-spot leaves than elsewhere, extending as an irregular line directly through the centers of initial spots.

Of the various groups of insects suggested to the writer by Dr. Illings-

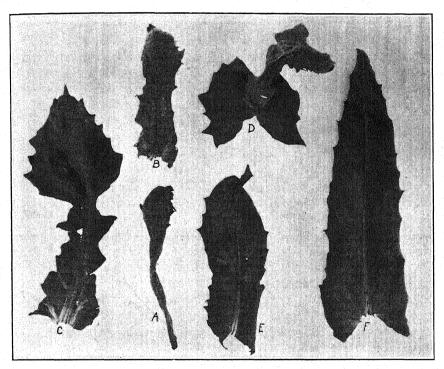


Fig. 4. Yellow spot in *Emilia sagittata* from the field, showing different degrees of mottling and distortion. Normal leaves of this plant are highly varied in shape but all of these leaves except that at the right are somewhat deformed. A is chlorotic throughout; B has a few green islands on a chlorotic background; C, D, and E are partly affected; and F has only a small area slightly mottled near its apex.

worth, only one, the thrips, was able to qualify as to size.³ Examination of thrips eggs in onion leaves, both in surface and sectional views, revealed striking resemblance in size and form. Finally, pineapple leaves, collected

³ That *Triphleps* sp., suggested by Illingsworth, was not the vector was shown by the fact that its eggs are much too large to fit specifications. (Illingsworth, J. F. An Anthocorid (Triphleps sp.). U. S. Dept. Agr. Bur. Entom. Insect Pest Survey Bul. 10: 24. 1930. (Mimeographed.)).

by Mr. Paxton from an infested area, were found to contain eggs not yet hatched. These were observed under a binocular microscope and, as they hatched, thrips larvae were seen to emerge from apertures that were typical in all details of the ovipunctures associated with initial spots. Thus it became almost certain that the vector of yellow spot was a species of thrips.

Search for another host plant. Even before the probable vector had been found a search was begun for a weed host of the yellow-spot virus. Evidence from the field occurrence of the disease suggested that whatever the vector might be, it was probably breeding on some infected plant other than pineapple. The suddenness with which newly set fields may become heavily infested suggested a migration of the vector from outside the field, and the standard practices of pineapple culture were such that diseased pineapple plants in near-by fields were too rare to serve as the source of infective insects at the beginning of an outbreak.

Several species of weeds in and around pineapple fields exhibited symptoms of viroses, chiefly of mosaic type, but a disease of Emilia sagittata (Vahl) DC.4 appeared the most closely related to the pineapple yellow spot in symptomatology and in distribution with respect both to space and time. Symptoms on this plant (Figs. 4 and 5) involved, in addition to mottling, a distinct tendency towards ring spot with zonate, circular, chlorotic patterns that suggested the disease in pineapple. Moreover, although Emilia was abundantly distributed over Oahu, it was diseased chiefly in those areas where the pineapple disease was abundant, and was found diseased throughout the range of the pineapple disease on Oahu and Maui.⁵ Furthermore, in a field where yellow spot had recently appeared, there were few diseased Emilia plants on October 17, 1929, but the percentage of infected Emilia increased rapidly during the following weeks. For these several reasons, the disease of Emilia was regarded as probably identical in cause with yellow spot and diseased Emilia was used in various experiments during the search for a vector.

This disease in Emilia, which will be described more fully in a later paper, is characterized by extreme mosaic symptoms in the first few leaves produced after inoculation and by a mild circular blotching in later leaves, which sometimes develops into an extremely involved pattern of concentrically zonate lines of dark and light green (Figs. 4 and 5).

4 This plant was listed earlier (5) under the synonymous name, *Emilia flammea* Cassini, following the usage of Bailey (1). Specimens collected locally were compared with the type of *Cacalia sagittata* Vahl through the courtesy of Drs. Harold St. John and S. F. Blake, with the result that the name *Emilia sagittata* (Vahl) DC. appears to be the correct form.

⁵ Since that time it has been found in the diseased condition coextensive with the pineapple disease on 3 islands but not, as yet, on Lanai and Hawaii, where yellow spot is very rare.

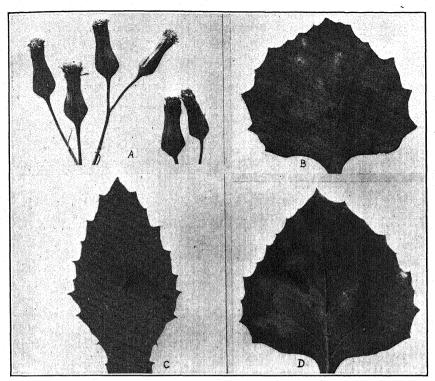


Fig. 5. Varied symptoms of yellow spot in *Emilia sagittata*. A. Mosaic mottling in the form of dark and light green stripes on involucres of flower heads. This develops only immediately following infection. B. Extreme development of zonately banded chlorosis in an old leaf. C. Dark necrotic line extending in zigzag course across the leaf. The line was opaque but not colored. Below it, at the left of the midrib, note a corresponding whitish line. This leaf was below the first mottled leaf on the plant. D. Mild mottling without distortion, a condition often seen on plants in the greenhouse.

Proof that Thrips transmit the virus from Emilia to pineapple. A species of thrips was commonly found on the leaves of diseased Emilia plants. Therefore, when it was learned from the study of insect punctures that thrips were the probable vectors, thrips were collected from diseased Emilia plants in the field and liberated on seedling pineapple plants in individual plant cages in the greenhouse. Table 1 shows the results of these and other preliminary tests. As indicated in the table, 21 of the 50 pineapple plants so treated developed yellow spot. Subsequent tests, some of which are reported in table 2, have shown diseased Emilia plants from the open field to be a reliable source of viruliferous thrips, subject to certain exceptions set forth in the discussion. Moreover, when similar thrips from diseased Emilia were caged upon healthy Emilia, these plants developed

TABLE 1.—Preliminary inoculation experiments with Thrips tabaci, adults and larvae, collected on various plants in pineapple fields or intercycle areas and liberated on test plants in individual plant insect cages

Insects			Test	plants			
	Applied to each	Pin	eapple	$Emilia\ sagittat$			
Sources	plant	Tested	Infected	Tested	Infected		
Emilia sagittata	1 to 10 ad.b & l.b	29	10				
E. sagittata, diseased	5 ad.	4	3				
	10 "	3	1		1		
"	20	10	5	1 - 1 1 - 1 - 1 - 1 - 1 - 1 - 1 - 1 - 1			
	5 '' & l.	2	1	12	7		
	10	2	1				
	1 to 3 l.			4	3		
Emilia sp. (orange), diseased	3 ''			1	1		
Senecio hieracifolia	1 ad.	4	2	3	2		
Bidens pilosa	20 ''	10	7				
Radish flowers, Raphanus sp	30 "	10	6				
	10 "			5	3		
	5 "	5	0				
	20 ''	5	0				
Mustard flowers, Brassica sp	5 ''	5	0		1 1 1		
Jack bean, Canavalia ensiformis	5 ''	6	0		27 144		
Pea, Pisum sativum	5 ''	10	0	32	0		
	2 to 4 ''			28	0		
Sonchus oleraceus	4 1.	1	1	3	1		
"	ad. & 1.	3	2	2	2		

a Thrips chiefly from leaves unless flowers are specified.

characteristic symptoms (table 1), and, although not shown by the table, when thrips reared through several generations on such diseased Emilia plants were transferred to pineapple seedlings, the typical yellow-spot symptoms developed. Several collections of these thrips have been determined by Dudley Moulton as the common onion thrips, *Thrips tabaci*, an insect that formerly had not been reported from the pineapple.

These preliminary tests showed that thrips from diseased Emilia were able, in some way, to cause the same disease in Emilia and yellow spot in pineapple. Also, they showed that thrips from certain other plants, even those not showing any recognized symptoms of virosis might do similarly, while thrips from some other plants might not. Contradictory results with different collections from radish flowers from the same field, collected at different times, emphasized the fact that the mere collection of infective thrips from a certain host plant might not indicate the true source of the

b Abbreviations: ad. = adults, and l. = larvae.

TABLE 2.—Virus transmission by thrips collected from diseased plants of Emilia sagittata from the same intercycle field on different dates

Insects		Test plants									
		Р	ineapple		E	. sagitta	ta				
Collection date	Number		Inf	ected		Infe	ected				
	per plant	Tested	Num- ber	Per cent	Tested	Num- ber	Per cent				
October 1, 1930a	1 adult	10	1	10			11.4				
	5 adults	10	2	20		1. 1. 1. 1. 1. 1. 1. 1. 1. 1. 1. 1. 1. 1					
	25 "	10	3	30							
	5 larvae	10	1	10	10	10	100				
(Reared from pupae from				* ***							
Oct. 1 collection)	5 adults	5	5	100	5	4	80				
December 12	10 "	38	32	84							
17	10 "	49	39	80							
21	10 "	19	15	79							
28	10 "	27	22	81							
30	10 "	43	35	81							
January 2, 1931	10 "	31	27	87							
5	10 ''	33	32	97							

^a On this date many Emilia plants in this field were healthy and the infected plants were chiefly in early stages of the disease as shown by localization of symptoms on the younger leaves. On the later dates almost all of the larger Emilia plants had long been diseased.

assumed virus, since adult thrips may move from plant to plant and even the larvae may possibly do so where plants of the different species are in close contact. Consequently, it was recognized that infective insects from some of these plants may have acquired the virus while feeding on Emilia or other plants.

DETAILED STUDIES OF VIRUS TRANSMISSION

To determine more fully the rôle of *Thrips tabaci* in the transmission of yellow spot and to provide a sound basis for virus-host studies, a detailed investigation of transmission was undertaken. This involved preliminary work in the development of suitable techniques of rearing, handling, and testing insects. Since only an open-sided greenhouse was available for this work, special attention had to be given to the question of insect cages.

Stock colonies of thrips. Colonies of infective thrips were maintained readily at all times on diseased Emilia plants in the individual plant cages described below.

Noninfective colonies for use in these studies were started from two sources by a method similar to that of Stahl and Carsner (8), as follows:

Adults from infective colonies on Emilia and adults from a field collection from peas, *Pisum sativum* L., were allowed to deposit their eggs in leaves of healthy Emilia and of pea. After 4 days the eggs were subjected to observation under a binocular microscope and, as they hatched, the larvae were transferred immediately, before they fed, to separate seedlings of Emilia and of pea. In this way 13 individual colonies, each of which developed from a single larva, were established. All of these colonies, when tested both on pineapple and on Emilia, have proved consistently noninfective. Only a few were retained, and this work has been done chiefly with one of them.

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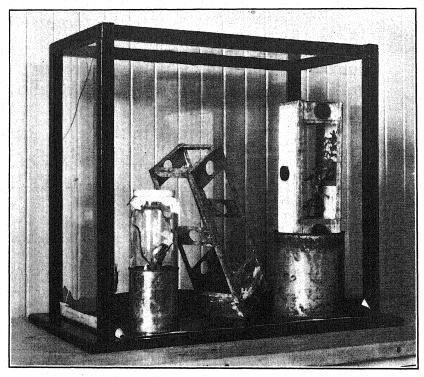


FIG. 6. Transfer chamber, individual plant cage, stock colony cage, and the galvanizediron frame of the latter, as used in experiments with thrips.

Insect cages. The stock colonies have been maintained in special insect cages constructed, as shown in Figure 6, of galvanized iron, clear sheet pyraline, and a heavy grade of white cotton broadcloth, over pea seedlings

⁶ Thrips tabaci is parthenogenetic in its reproduction, and all or nearly all of the individuals encountered in such colonies have been females. Thus there has been no difficulty in rearing colonies from single individuals.

grown in gallon canners' tins (No. 10) of soil. Rubber stoppers, size 6, fitted into smooth-cut holes in the metal, made other openings unnecessary. The metal base of the cage was thrust about 2 in. into the soil. The surface of the soil was then sealed around the plant stems by pouring a layer of soft wax (paraffin wax, 5 parts; white petrolatum, 2 parts; and beeswax, 2 parts, by weight). This wax was covered with a layer of air-dry, fumigated soil to provide a suitable place for pupation. Water was applied to the soil outside of the cage and did not wet the dry soil within.

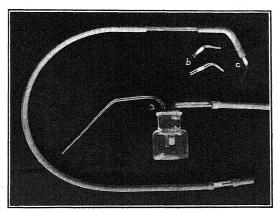


Fig. 7. Apparatus for handling thrips. a. Suction apparatus for collecting thrips into a bottle. b. Small glass pipettes plugged with cotton. c. A similar pipette in a suitable holder of glass and rubber tubing.

Under these conditions thrips reproduce rapidly on peas and monthly transfers to fresh colony cages have been necessary. With a start of 15 adults on 4 plants, it is customary to obtain over 100 adults and many larvae by the time the plants are killed after a month or 6 weeks. For still larger colonies, where less precision was required, large cages of somewhat similar construction to those described above have been used over wooden flats, dispensing with the wax seal.

For individual-plant inoculation chambers, wide-mouth glass jars of 1-qt. capacity were used. The bottoms of these were removed with an electrically heated resistance wire, and the tops were covered with white broadcloth. These jars were pressed into the soil in the quart-size (No. $2\frac{1}{2}$) canners' tins, which were used as plant containers (see Fig. 6).

Handling the insects. Suitable methods of handling thrips were adapted from standard entomological techniques as follows: Suction apparatus of various types was used extensively. For collecting thrips into bottles or vials for various purposes, the apparatus shown in figure 7, a, was very useful when connected with a suction line or with the mouth.

Small pipettes (Fig. 7, b), plugged with cotton were used extensively in transferring adults. The desired number of insects was drawn into the pipette and then the pipette itself was slipped out of the rubber tube and placed in the appropriate insect cage where the insects were allowed to emerge. This proved more satisfactory than forcibly expelling the thrips from the pipette. Larvae and sometimes individual adults were transferred with the moistened tip of a fine brush.

All insect transfers were made in a laboratory room some distance from the greenhouse. The actual transfers were made over black oilcloth in the open-front transfer chamber (Fig. 6), which was thoroughly cleaned between transfers of different lots of thrips.

Plant materials. The pineapple plants used in these tests were grown from seed (chance seed of Cayenne variety) in covered dishes and transplanted into individual plant chambers in time to resume growth before use. The Smooth Cayenne variety is heterozygous and its seedling progeny are highly varied in character. This explains certain variations in the behavior of individual plants.

Emilia sagittata was propagated both from seed and cuttings. Seedlings start slowly and, in the dim light of insect cages, are slender. Stem cuttings of older plants strike root rapidly and provide stronger plants. Propagating materials were handled both in large metal-frame cages over flats and in individual-plant cages. Throughout the greater part of this work soil was subjected to heavy applications of carbon bisulphide, since both Emilia and peas were found to grow very poorly in steam-sterilized soil.

The pineapple plant, even as a young seedling, appears relatively unfavorable for the growth and reproduction of *Thrips tabaci*, and irregular results were obtained unless 5 or more vigorous thrips were applied to each plant. Consequently, *Emilia sagittata*, which is a favorable host plant, has been used extensively. Young larvae, when transferred to it, will mature and reproduce. Symptoms are as characteristic in Emilia when well developed as in pineapple. In the later work, therefore, test insects were applied first to healthy Emilia. If the symptoms that developed were at all uncertain, these thrips or their progeny were later transferred from these Emilia plants to pineapple. Development of the characteristic disease in pineapple was thus the ultimate criterion in these tests.

EXPERIMENTS

The more significant experiments bearing upon virus transmission by thrips are summarized in tables 3 and 4. Insect-free test plants, although not indicated in these tables, were used freely throughout this work, and none of them became infected. Also, though not indicated in the tables,

thrips from test Emilia plants in many of these experiments were transferred to pineapple seedlings to confirm the positive or negative indications.

Experiments with adults. Before the special noninfective colonies of thrips were available, experiments were begun with thrips from other sources. Earlier tests (Table 1) had shown thrips from peas and from Jack bean to be noninfective, so these were used in experiments 1 and 2 of table 3. Adults were fed upon leaves of diseased pineapple and Emilia, with proper checks, and then transferred to test plants. Over 150 adults were exposed to diseased leaves for 1 to 4 days, but none transmitted the virus.

When the pedigreed noninfective colonies, reared as described above, were of sufficient size, thrips from them were used in further experiments (4, 5, 6, and 7 of table 3). Adults were confined for different lengths of time upon excised diseased leaves of Emilia and pineapple, in small widemouth bottles covered with broadcloth. After this period of experimental feeding the insects were liberated upon the test plants where, in the case of Emilia at least, they fed freely and reproduced. Even after the 11- and 10-day periods of experiments 4 and 6, respectively, the thrips fed vigorously upon the test plants. The consistent failure of transmission in these tests and in others of a minor nature not tabulated was extremely puzzling.

Experiment 7 of table 3 was a notable exception in that very satisfactory transmission to both Emilia and pineapple was obtained. As far as was recognized at the time, this experiment differed from the others of table 3 only in the somewhat longer period of exposure to diseased leaves, and the difference between the 10 to 11 days of other tests and 14 days of this one did not appear significant because the experimental thrips were allowed to remain on the test plants after transfer. After repeated failure to duplicate these results, it was recognized finally that, in this test, larval progeny of the experimental insects were probably transferred to the test plants.

Experiments with progeny. Experiments that utilized a different method indicated the probable difficulty. Insect-free plants of diseased Emilia, grown vegetatively from 1 plant, were set into separate inoculation chambers and thrips from a noninfective colony were placed upon them to feed. After 4 weeks, when thrips were transferred to test plants, 5 of the 8 colonies proved infective. The duration of exposure to diseased Emilia was such that the progeny had had time to come to maturity. Consequently, it appeared probable that transmission was by the progeny rather than the parent insects.

To test this, experiment 1, A to D, of table 4 was organized more precisely along similar lines. Four mildly blotched, thrips-free cuttings of 1

TABLE 3.—Experiments with adult thrips showing their failure to become infective

	Pineapple	Infected	0				1 1	0	00	0	00	0	0	0	0	0	0	0	9
Test plants	Pine	Tested	1.0					15	12 9	9	א מז	o 10	ເດນ	o 10	က	∞	4	7.5	10
Test 1	Emilia	Infected	С	00	C C	o C :	00	i	0	0	00	0	0	00	0		0	С	∞
management of the control of the con	Em	Tested	90	75 9	10	2 10	10 2	-	l _{re}	4	זט ת	. ro	ic i	ດາຕ	က		ಣ	ıc	10
	Applied to each	plantb	5 nd.			1 H	1 to 4 ''		10 rc		ron S) 10)) <u>.</u>	4 to 5 "	; £	4 to 5e "
	lg	Duration	(Days)	c) c)	m C	» П (s) 4	0	നന	· 60	es [-	33	ו מו	~ G	11,	4 9	~~~ 	,	14
Insects	Experimental feeding (adults only)	Exposed to—	Oa	Healthy pineapple Diseased	Emilia	sed E))))	0	Diseased pineapple Healthy	Diseased "	Healthy Emilia	D.seased Emilia	99	3)		(Diseased Emilia then healthy Emilia	Diseased pineapple	0	Diseased Emilia
	Source		Peas, open field	37 37 33	Jack bean field	(reared on peas)	"	"	Stock colony	, ,, ,,	"	"	"	3)))	22 23	33 33 33 33	9 . 9	"	, , , , , , , , , , , , , , , , , , , ,
	Experiment number				© 1				ന		4					ນລ	9	7	

^a Transferred directly to the test plants with no experimental feeding.
^b Where two numbers are listed, the smaller generally represents thrips transferred to Emilia, the larger to pineapple.
Adults are represented by ad.
^c Probably both adults and their larval progeny; no record on this point.

diseased Emilia plant were grown to large size in colony cages and 50 or more adults from noninfective colonies were added to each. After 5 days, adults were removed to test plants. After 19 to 20 days large larvae were removed and tested. These were the progeny of the adults introduced. From all 4 colonies the adults were noninfective. Larvae were tested from only 3 (plant C died early), and from 2 of these colonies they proved highly infective. Note that of 24 Emilia test plants that received larvae from D, 23 became infected.

Experiments 2A, 2B, 3 and 4 of table 4, were of similar nature, comparing adults with their larval progeny reared on 3 other hosts of the yellow-spot virus. Consistently, the adults from noninfective colonies remained noninfective after a period of feeding upon the diseased plant, while their progeny became infective.

Experiments with larvae. Experiments 2C, 5, 6, and 7 of this same table represent a different procedure in that thrips larvae from noninfective colonies on peas were fed in bottles on excised leaves of the infected plants. In each instance, larvae became infective after even 1 day's exposure to the source of virus. In experiments 5 and 7 of table 4, special care was taken to eliminate other variables. The adults used were from a young colony and most, if not all, were young adults. The larvae were collected from the same individual pea plants as were the adults. The several leaves used as the source of virus were each split in half, 1 half going to the larvae, the other half to the adults. The results of these experiments, therefore, in addition to the ample evidence of the others of tables 3 and 4, show conclusively that larvae from noninfective colonies acquire the virus readily by feeding on infected plants of different species but that adults, treated similarly, do not.⁷

Retention of virus through pupation. At the same time it is plain that thrips that have acquired the virus as larvae retain their infective capacity as adults. This is shown by experiment 5 of table 4, in which some of the larvae were allowed to pupate and later, after maturing, were tested as adults and found infective. The same situation is revealed by the experiment with field-collected insects reported in table 2.

Transmission by larvae. Since, in studies reported above, the thrips were allowed to remain indefinitely upon the test plants where, in the case of Emilia at least, larvae developed into adults, these experiments did not show whether larvae, as such, actually transmit the virus. The general association of oviposition marks with initial spots in the field suggests that

⁷ Since obtaining these results the writer has learned by conversation with Dr. Geoffrey Samuel, while he was in Honolulu, that he has come to similar conclusions with respect to the transmission of the tomato spotted-wilt virus by *Frankliniella insularis* in Australia.

TABLE 4.--Experiments comparing adults and larvae in their ability to become infective

		Insects				Test	Test plants	
Experiment		Experimental feeding			F		, E	3
	Stage	Source of virus	Dura- tion	Appnea to each planta	Tested	Emilia d Infected	Tested	rineappie ed Infected
			(Days)					
1 A	Adult	0	0	5 ad.	ro	0	ıc	0
	,	Emilia	വ	2 to 5 "	9	0	က	0
	Eggb	33	20	31.	10	8	:	
В	Adult	0	0	2 to 5 ad.	20	0	2	0
	,	Emilia	29	2 to 5 "	20	0	က	0
	,,,		50		:	:	80	0
	$\mathbf{Egg^b}$		20c	31.	4	0		:
O	Adult	0	0	3 to 5 ad.	5	0	ည	0
	,,	Emilia	29	2 to 5 "	75	0	4	0
Q	"	0	0	3 to 5 ad.	5	0	ນ	0
	$\mathrm{Egg^b}$	Emilia	19°	31.	24	23	1	:
	,,,) () () () () () () () () () (19	5 to 81.	:		က	က
2 A	Adult	Richardsonia scabra	ъ	3 ad.	4	0	;	:
	Eggb	, , , , , , , , , , , , , , , , , , , ,	140	41.	ಬ	2		:
В	Adult	"	ນດ	3 ad.	4	0		:
	Eggb	,,	14	41.	10	ıc	1	:
ರ	Larva		c ₁	41.	ນ	ro	:	
က	Adult	Ageratum	0.1	5 ad.	4	0	4	0
	Egg^{b}	"	18c	2 1.	©1	©1		:
4	Adult	Stachys	ນ	3 ad.	4	0		:
	. , , ,	, , , , , , , , , , , , , , , , , , , ,	14	33 66	ıc	_	-	

TABLE 4.—(Continued)

nts	Pineapple	Tested Infected	; ;	o °	6	ာ	0		1		1	1			0	
Test plants	Emilia	Tested Infected T	က	0	0	0	4	20	10		9	0		0		4
	pplied to	each planta Test	41.	5 ad.	3 to 10 '' 3	3 to 5 " 5	3 to 51.	1 or 2 ad. 5	41. 11	41.	41.	3 ad. 5	3 p. 5	3 ad. 5	2 to 10 ''	3 p. 5
	A	Dura- ea tion	2	0	1 3		1 3	17d 1	-	H	—	23	63	67	62	63
Insects	Experimental feeding	Source of virus	"	0	Emilia	99	u	33	•	7,7	,,	Pineapple	M. 1 1 2 29 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	,		•
		Stage	Larva	Adult	"	**	Larva	•))	**	,,,	Adult	Larva	Adult	"	Larva
	Experiment		4 (cont.)						ď			7 A		щ)	

a Where 2 numbers are listed, the smaller generally represents the thrips transferred to Emilia, and the larger to pineapples. Adults are represented by ad., larvae by 1, and pupae by p. b Thrips hatched from eggs deposited in the diseased plant, source of virus, and reared thereon. This represents the period from liberation of parent adults on the virus source to the transfer of larval progeny

to test plants.

The matured adults fed only upon healthy Emilia d These insects fed on the virus source until they pupated. prior to being tested. the pineapple, at least, is inoculated generally by adults. Special tests were made, therefore, with larvae confined in cotton-plugged glass capsules upon individual leaves of Emilia and later recovered from the plants. Four plants, thus inoculated, developed the disease, proving that larvae do transmit the virus.

Incubation period in the plant. During the experiments outlined above and others not reported in detail, observations of test plants have been made, generally, at intervals of 3 days. In some tests they have been more frequent at the outset to determine the minimum incubation period with accuracy. Data collected in this way (Table 5, line C) indicate a minimum

TABLE 5.—Incubation periods in pineapple, Emilia, and Thrips tabaci. An incubation period in the insect is indicated by comparison of apparent incubation periods in Emilia plants inoculated by insects of different histories

m	M1-11-	Apparer	it incubation	period
Thrips used in tests	Test plants	Minimum	Maximum	Mean
A CR: 6 - 3-14 - 6		Days	Days	Days
A. Chiefly adults from diseased plants	161 pineapple seedlings	7	27+	12.2
B. Larvae fed one day				
on infected plant and left to ma-				
ture upon the test				
plant	48 Emilia sagittata	18	35	24.9
C. Large larvae and adults reared on				
infected plants	82 " "	8	27	14.6
Indicated incubation period	l of virus in thrips (B			
minus C)	* ``	10	8	10.3

incubation period in Emilia of 8 or 9 days and a mean period of 14.6 days calculated on the basis of 82 plants. The approximate maximum of 27 days is not very significant, owing to less frequent observation at the end of an experiment, and, similarly, the mean of 14.6 days is probably too great as a consequence of not making daily observations throughout. Part of the variation in apparent incubation period is a result of different rates of growth, since symptoms in this plant depend for their expression on the development of new leaves from the growing point.

The incubation period in the pineapple is less readily determined with accuracy, because the initial symptoms develop at the point of feeding and because the feeding of even noninfective thrips may cause a conspicuous local yellowing. Here, again, the rate of growth enters as a factor, because

thrips inoculate the pineapple leaf near its base, where other leaves hide the symptoms until the leaf elongates from below, lifting the initial spot to view. The observed incubation periods in 161 plants are shown in table 5, line A. Seven days was the minimum and 12.2 days the mean. Again, as with Emilia, this mean is perhaps slightly high because observations were not made daily. More limited observations on young plants grown from crowns in the field indicate the incubation period to be about 2 weeks.

Incubation period in the insect. In the course of these studies it was clearly apparent that when Emilia plants were inoculated by thrips reared on diseased plants, symptoms developed in fewer days than when inoculation was by thrips that had fed as larvae for only 1 day on a diseased plant prior to being placed on the test plant. Table 5 compares the minimum, maxi-

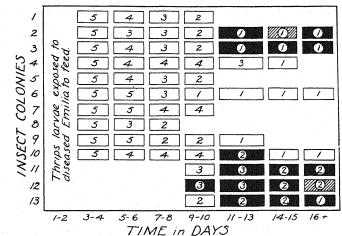


Fig. 8. Incubation period of virus in *Thrips tabaci*. Rectangles represent individual test plants on which thrips fed during the days indicated below each column. The number within each represents the thrips placed upon the plant. Black rectangles represent infected plants, and shaded rectangles plants that died. The declining numbers of insects in each colony represent loss of thrips chiefly by death. Three of the infected plants were inoculated by larvae, the others by adults.

mum, and mean periods, in days, between adding the insects and observing symptoms in 82 and 48 plants inoculated in these two ways. Assuming the actual period of incubation in the test plants to be the same in both cases, the difference between the apparent periods must be a measure of the length of incubation period in the insect. These results suggest, on this basis, an incubation period of the virus in thrips of about 10 days. Kunkel (3), working with aster yellows, found that the incubation period in the plant may be shorter when inoculation is by an insect that has carried the virus a long time than when by an insect that has just become infective. Possibly, therefore, the incubation period in thrips may be somewhat less than

10 days. The use of different numbers of insects in these tests did not invalidate results, since the shortest incubation period observed, 8 days, and several other short periods were observed following inoculation by a single adult.

A more direct test for incubation period in thrips is reported in figure 8. Thrips larvae of mixed ages were transferred from a noninfective colony on peas to an excised leaf of diseased Emilia. On the 3rd day, 5 of these larvae were confined in single-leaf cages upon each of 10 healthy Emilia plants. After 2 days they were removed from these plants and caged upon others, and so on to the 16th day, when the insects that survived were liberated on Emilia plants for the rest of their lives. On the 9th day, 3 additional colonies were started with adults reared on excised leaves of healthy Emilia from larvae that had fed on diseased Emilia the same as colonies 1 to 10. Some colonies died out entirely, some insects died in all colonies, and 2 test plants died without exhibiting symptoms. It will be seen from the figure that 1 of the plants, fed on from the 9th to the 10th day, became infected. The first transmission by the other colonies was to plants fed on from the 11th to 13th day.

The results of this test indicate, in confirmation of the above evidence, that thrips are noninfective until approximately 10 days after they first feed, as larvae, upon an infected plant. No more precise determination has been attempted. It must be recognized that this period is close to the maximum duration of the larval stages under favorable conditions and that the intervention of the pupal stage may be a factor in lengthening the apparent period of incubation in the insect. Still, in this test, 3 plants were inoculated by either large larvae or prepupae. Clearly, no close interpretations are permissible until more detailed studies are carried out with closer attention to the insect itself.

DISCUSSION

Among the insects that transmit plant viruses the Thysanoptera (thrips) have received consideration only recently. Several workers have suggested that species of thrips might be vectors of different viruses, but the present case appears to be only the second one supported by adequate experimentation. The report on spotted wilt of tomato by Samuel, Bald, and Pittman (7) indicates several points of similarity between the 2 viruses and their relation to their vectors.

The method of finding the vector appears particularly interesting in that *Thrips tabaci* was not formerly known ever to feed upon the pineapple.⁸ Even now it appears to inoculate the pineapple only incidentally

⁸ Although a prelminary report of the Australian work with thrips had already been published by Pittman (6), this was unknown to the writer until he had found, by independent means, that *Thrips tabaci* is the vector of yellow spot.

when it has left its preferred hosts. On some young pineapple seedlings in the greenhouse a new generation will develop, but, even here, where conditions favor rapid reproduction on suitable host plants, a colony of thrips usually disappears within 10 days or less. Eggs that are laid in the leaves hatch and the young larvae feed for a time, but it seems probable that this species of thrips seldom comes to maturity on young pineapple plants in the field.

Clearly, the pineapple is not a suitable colony plant for rearing thrips. Furthermore, since pineapples are quickly killed by the disease, they are not a satisfactory source of the virus for experimental use. It would have been very difficult to carry on these studies of transmission were it not for the early recognition of a more suitable host of both virus and thrips in *Emilia sagittata*.

As indicated in table 4 and as will be discussed more fully in a later paper, several other hosts of the virus have since been found, but this species of Emilia still appears to be of the greatest significance in the occurrence of pineapple yellow spot. Not only is it a favored host of the insect and highly susceptible to infection, but it lives long in the diseased condition and thus may maintain a colony of infective thrips for several months. Moreover, it is one of the most common weeds in pineapple fields, in intercycle fields, and in nongrassy waste lands in those areas where yellow spot is prevalent. Almost pure stands of it may develop in such areas, producing a great population of viruliferous insects.

Were it not for this and other hosts of the virus, the pineapple yellow spot might be self-exterminating, since the pineapple plant infected with yellow spot soon dies. Consequently, transmission of the virus in diseased planting material within an infested region is not of serious importance and roguing is not needed as a control measure. Dead and dying plants are removed from fields simply to make space for replants. It is the movement of infective thrips from other hosts of the virus that accounts for the appearance of this disease in the pineapple.

From the standpoint of dissemination of this virus it is significant that noninfective adult thrips, in migration, may feed upon diseased host plants and then move off to other plants without transmitting the disease. It is the progeny of these adults, grown to maturity on infected plants, that will transmit the disease when they in turn move to other plants. As shown by table 2, noninfective adults may be collected from recently infected host plants.

There is no reason to think that this is the only species of thrips that may transmit this virus. Other species have not been tested. Since, how-

⁹ The incomplete list of hosts includes members of the following families: Bromeliaceae, Liliaceae, Caryophyllaceae, Leguminosae, Labiatae, Solanaceae, Rubiaceae, and Compositae.

ever, *Thrips tabaci* is the most commonly observed species on the known hosts of the virus it seems clear that it is the chief vector. In the case of spotted wilt of tomato (7), the known vector, *Frankliniella insularis*, is absent from part of the range of the disease, indicating that at least a second vector must be involved. That insects other than species of thrips do not transmit to the pineapple often, if at all, is indicated by the close association of thrips ovipunctures with initial spots.

Control of yellow spot in pineapple thus resolves itself into the problems of reducing the reservoir of the virus in wild hosts, controlling the insect, and breeding resistant varieties of pineapple. Of these, thrips control offers most promise of early results. This is being studied by the Entomologist. Marked differences in the behavior of the heterozygous pineapple seedlings used in these studies indicate that significant resistance may be found.

The detailed studies of transmission are of peculiar interest in that they demonstrate a specific relationship between virus and insect in the case of a disease that appears, on the basis of symptoms, to be more closely related to the mosaic than to the yellows type of disease. As has been suggested by Kunkel (3, 4) in the transmission of aster yellows by *Cicadula sexnotata*, the presence of an incubation period of the virus in the insect seems to support the view that the virus is in the nature of a living entity rather than merely a chemical. The incubation period in *Thrips tabaci* is longer than seems to be recorded for any virus except that of aster yellows. Its close approach to the total duration of the larval stages, together with the fact that larvae only are able to acquire the virus, complicates the present case and indicates the need for a further study of the larval stages separately.

It is only possible to speculate upon the mechanism that prevents adult thrips from becoming infective, even though thrips that have earlier acquired the virus as larvae may transmit freely in their adult stage. Clearly, the feeding of infective adults introduces the virus into the plant in a manner suitable for infection. Moreover, adults doubtless ingest the virus during their feeding on diseased plants. The difference appears to lie in some factor that prevents the virus from moving out of the digestive tract and into organs from which it may be ejected during feeding. Some fundamental change in the physiology of the insect seems indicated. Possibly, this is a change in digestion such that, in adults, the virus is promptly destroyed, whereas in the larvae it is not harmed but is able to migrate from the digestive tract to other organs. Or the difference may result from a change in the insect that prevents migration of the virus to the salivary glands or other parts from which it may be ejected.

SUMMARY

This paper presents in more detail the work summarized in a brief former note and reports further studies dealing with transmission of the virus of pineapple yellow spot by *Thrips tabaci*.

The identity of the vector, an insect not formerly reported from the pineapple, was inferred from a study of its oviposition marks associated with the initial stages of this disease.

A weed host of the virus, *Emilia sagittata*, which also is a favored host of *T. tabaci*, was recognized because of similarities of symptoms and of space and time distribution.

Thrips reared on this plant were found to transmit the virus to both Emilia and pineapple.

For detailed studies of transmission, noninfective colonies of thrips were reared from single larvae.

Methods of rearing, handling, and testing thrips used in these studies are described in full. Since the pineapple is not a suitable colony plant, Emilia and garden pea have been used extensively.

These studies have shown the following:

Larvae from a noninfective colony become infective after feeding upon a diseased plant, but adults treated similarly do not. The virus survives pupation, and insects that feed on a source of virus while larvae may be infective as adults.

There is a period of approximately 10 days after first feeding on a diseased plant before thrips transmit infection.

A single insect, larva or adult, may transmit the virus to Emilia.

The minimum incubation period in *E. sagittata* is about 8 days and the mean about 15 days. In young pineapple plants the minimum is 7 days and the mean about 12 days.

Thrips tabaci transmits the virus to and recovers it from several other plants in addition to pineapple and Emilia. These are to be considered in a separate paper together with symptom studies.

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THE DEVELOPMENT OF THE ROOT-KNOT NEMATODE IN RELATION TO ROOT TISSUES OF PINEAPPLE AND COWPEA¹

G. H. GODFREY AND JULIETTE OLIVEIRA

INTRODUCTION

There is need for more detailed information than has been available on the life history of the root-knot nematode, Heterodera radicicola (Greef) Müller, in relation to root tissues. This need is manifested by the lack of agreement between early investigators with regard to certain life-history details and by the lack of definite information on other points. These studies have clarified to some degree the details of time and manner of penetration of the larvae, length and characteristics of the motility period within the roots, orientation within the root tissues, relation of developmental stages to roots, and, finally, the time and duration of egg development. This graphic presentation of the main developmental stages of the nematode shows clearly the nature of the things that are happening within the root during the development of the nematode gall.

LITERATURE REVIEW

The first mention of root knot in the scientific literature was by Berkeley (2) in 1855. Though the causal organism was not described in any detail, the illustrations, both of the type of the disease and of the organism related to it, leave no doubt as to its identity. Since then, scores of papers have been written on the subject. The literature cited by Bessey (3) in 1911 is nearly complete in the way of reference to the early papers. We shall confine our references to those papers dealing strictly with our subject.

Atkinson (1) deals mainly with complex nematode galls, as he found them in the fields, and does not attempt a description of the relatively simple relationships existing in a primary infestation of a root. Frank (8, pp. 19-24) and Stone and Smith (20) add little to this, emphasizing mainly the complexities of the tissue relationships in the more advanced galls. Molliard (14) presents clearly a description with illustrations of typical multinucleate giant cells produced in the root tissues in the vicinity of the head end of the nematode. Tischler (21) describes in greater detail these giant cells with the histological details of mitotic and amitotic division of their nuclei. Bessey (3, p. 30) describes very briefly primary penetration

¹ Technical paper No. 27 of the Experiment Station of the Association of Hawaiian Pineapple Canners, University of Hawaii.

and arrangement of the nematodes in the tissues, stating that they may be either within the central cylinder or entirely in the cortex. In either case "the anterior end . . . is usually in close connection with the cells surrounding the conductive tissue." He mentions the fixation of the nematodes in the tissues and gives details regarding further development of the organisms. He refers to the host reaction as "hypertrophy of the tissue," the parenchyma cells becoming "abnormally large and multinucleate," referring to Tischler (21) as his authority. Occasional other investigators have made mention of this giant-cell formation without adding materially to the understanding of it. Byars (4) describes inoculation under pure culture conditions and describes initial penetration and initial host reactions from the point of view of external appearance. None of these writers presents accurately the picture of the position-and-time relationship of Heterodera radicicola to plant tissue, to gall development, and to reproduction: none differentiates clearly between the simpler primary reaction and the more complex phenomena that develop from secondary infestation Sengbusch (19), in studies on H. schachtii Schmidt, includes several photomicrographs of nematodes in process of development within root tissues and shows diagramatically their position in the roots in a case of general infection.

Many investigators give figures in round numbers as to length of life history, numbers of eggs produced, time required for hatching, etc. Reference will be made to specific papers in our discussion of these phases of the study.

METHODS

The general plan of procedure was to inoculate simultaneously a large number of root tips of two host plants, pineapple, Ananas sativus Schult., and cowpea, Vigna sinensis Endl., with Heterodera larvae, to excise the roots at intervals from time of the first penetration up to maturity of the nematode, and to preserve them for examination. As the first step, several pineapple plants were started in sterilized soil in the smaller root-observation boxes, developed at this station and described by Dr. Dean (6). Within 1 to 2 weeks after planting, roots were well started. Those in appropriate condition for inoculation are illustrated in an earlier paper by Godfrey (11, Fig. 5). Inoculation was made by applying at each root tip approximately 500 larvae produced by incubation of egg masses according to the method described by Godfrey (12), from cowpea roots such as are shown in that paper (Fig. 2).

With cowpeas the procedure was practically the same, preliminary experiments indicating the necessity for minor variations. The cowpeas were likewise planted in root-observation boxes. In 3 or 4 days root develop-

ment was sufficient for inoculation. Time of planting was adjusted so that roots of cowpeas and pineapples would be at a suitable stage for inoculation at the same time. For the entire period of observations inoculations of cowpeas and pineapple were made on the same date. Both lots of plants were therefore subjected to the same temperature range through the period of observations. Water applications were not weighed, but, in general, optima moisture conditions were maintained. Previous results by Godfrey (10) have shown that through the range of soil moistures favorable to plant growth, little, if any, difference in susceptibility obtains.

With both pineapple and cowpea plants 3 or 4 representative inoculated roots were cut at each of the following intervals after inoculation: 6, 12, 18, and 24 hours, and thereafter at day intervals. In every case they were gently washed, until free from adhering soil particles, with the aid of a camel's-hair brush. Killing was in warm (55° C.) Flemming's solution. The resulting instant killing of the nematodes was desirable in that it prevented them from going into unnatural contortions. The osmic acid of the Flemming's solution stained the nematodes black. By removing the roots from the fixative at the right time the root tissues were not over-The usual period was between 2 and 4 hours. If, occasionally, too great staining of roots occurred, a few minutes' exposure to strong hydrogen peroxide solution bleached them without reducing too greatly the color in the nematodes. The roots were then washed in running water over night, then dehydrated in graduated series of alcohols according to the usual methods, and cleared in clove oil. The resulting preparations showed to good advantage the growth stages of the nematodes and their relation to the root tissues. As a rule this material lends itself with splendid advantage to photographic reproduction. In many cases the whole root could be used for this. With thicker or more deeply stained roots it was sometimes advisable to slice off the upper and lower portions of the root in longitudinal parallel planes in order to show the stained nematodes to better advantage. Photographs were made with an 8 x 10 Eastman View camera with a special lens board adapted for a 48 mm. microtessar as the only lens. This was mounted on a Folmer and Schwing upright camera stand and had the additional provision of front and rear clamps to prevent vibration of the camera. In the illustrations herein presented magnification is 16 diameters throughout, except in cases specifically stated to be otherwise.

Permanent microscope slide mounts of the choicer materials chosen for photographing were readily made by mounting the materials directly from clove oil into relatively soft balsam and covering with cover glasses. As the balsam gradually thickened more was added at the edge until finally infiltration was complete. After several months the permanent sides still show the sharply delineated nondistorted nematodes in their natural position in the root tissues.

OBSERVATIONS ON PINEAPPLE

It should be noted that these observations apply only to the particular conditions of this experiment, which was conducted in the summer time. Unfortunately, temperature-control apparatus was lacking and our results cannot be stated in absolute terms, *i.e.*, in terms of stage of development of nematodes at a certain age and temperature.

A detailed chronological account of observations on pineapple roots is first presented. Following this, observations on cowpea roots are recorded with special emphasis upon the differences that were observable between the two plants.

In pineapple the first penetration of nematodes occurred some time prior to 6 hours after inoculation. The 6-hour observation showed that a few nematodes had already penetrated into the interior of the root tips. Between 6 and 12 hours heavy additional penetration occurred, as shown by figure 1, A to D, at 12 hours. It appears at this stage that mass action is

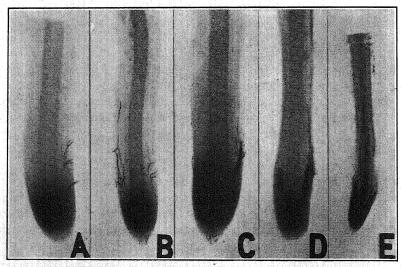


Fig. 1. Early stages of infection of pineapple roots with *Heterodera radicicola*. A to D. 12 hours after inoculation. E. 18 hours after inoculation. The roots are entire (not sections), killed in Flemming's solution, and cleared in clove oil. In A to D note larvae in the act of entering the roots. B and C show larvae entering through the root cap; C and D show evidence of mass activity on the part of the nematodes; E shows heavy infection of a small root. Magnification about 16 diameters.

contributory to penetration, for large numbers of larvae frequently penetrate the root on one side and at or near the same point. Some of the material showed a large number of larvae entering at one side and half in and half out of the root (Fig. 1, A, B, and C). Penetration takes place

mostly on the side of the root just back of the root tip but distinctly in the meristematic region. Occasional early infections show larvae actually at the tip of the root, in the act of penetrating the root cap (Fig. 1, B and C). The 18- and 24-hour material (Figs. 1, E, and 2, A) show continued root penetration.

At 48 hours or even earlier (Fig. 2, B) a semblance of root enlargement occurs. There is evidence that the abundance of infestation in the meristematic region results in retardation of growth of root meristem, since forward growth of the roots has been checked. Large numbers of cases have been observed in which heavily inoculated roots advanced scarcely a quarter of an inch in the first 2 days after inoculation, whereas noninoculated con-

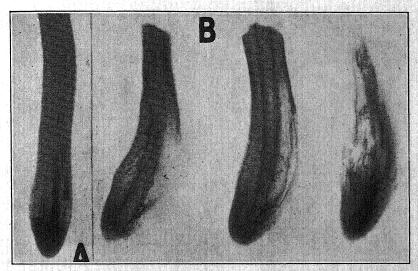


Fig. 2. Pineapple roots. A. 24 hours after inoculation. B. 48 hours after inoculation. A shows migration of the earliest invaders. Root B was sectioned into three parts to show more clearly the abundance of larvae in the root tip region; right, upper portion, middle, central portion, and left, lower portion of the root. Magnification of this and succeeding figures 16 diameters unless otherwise indicated.

trol roots a few inches away had grown 2 inches or more. This seeming enlargement cannot be true gall formation, however, for the larvae are not yet in the position in the roots that is necessary to the first development of true gall tissue. This will be explained in another paper on the histology of the early stages of gall formation. Even at 48 hours, in pineapple root (Fig. 2, B), the apparent swelling is due to retardation of forward growth of the root and abnormally directed growth of the weakened meristematic tissues rather than to true gall development.

These early stages (Fig. 2) all show marked concentration of nematodes in the tip region. There is evidence, however, of the beginning of directed

migration, for larvae can be seen in the cortex parallel with the stele, with their head ends approaching its periphery. In the meanwhile, maturation of tissues goes forward in the root tip and, with it, continued systematic orientation of the larvae. They advance through the cortical tissues and by the 4th day orient themselves typically (Fig. 3, A) with their head ends buried in the region of the clearly differentiated pericycle of the stele and endodermis of the cortex with their bodies extending diagonally outward and downward in the cortex. When once established in this position they begin to feed and immediately to grow. From this time on until maturity, with the male, and permanently, with the female, the position is the same.

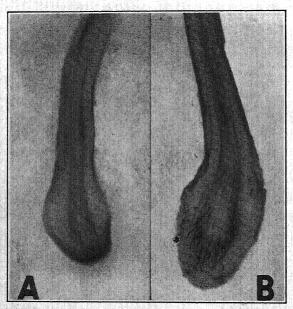


Fig. 3. A. Pineapple root 4 days after inoculation, showing orientation of the nematodes and early stages of gall formation. B. Another root 7 days after inoculation, showing the systematic orientation of the nematodes with their head ends embedded in the periphery of the stele.

Larvae were in no case seen thus permanently established within the central cylinder, as mentioned by Bessey (3, p. 30), and the writers doubt the occurrence of such arrangement in primary infections. No further migration and, in fact, very little movement of any kind occurs. An immediate host reaction takes place in the form of development of multinucleate giant cells in the immediate vicinity of the phloem vessels, as described by Molliard (14) and Tischler (21). Figure 3, B, at 7 days, shows to excellent advantage the orientation of the nematodes in the cortical tissues and suggests the early stages of developments responsible for formation of the galls.

Here some growth in the form of thickening rather than lengthening of the larvae has already occurred.

From this time on, for several days, growth of the nematode, in the pineapple roots, is rather slow and somewhat irregular. The nematode becomes typically fusiform, then as a result of a period of more rapid development, flask-shape, and finally spherical.

Heavy infestation of a pineapple root as a rule results in complete cessation of forward growth. Occasionally, however, after a period during which forward growth has obviously been checked, it is renewed by a re-

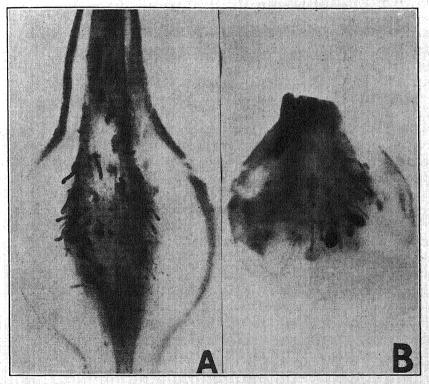


Fig. 4. A. Pineapple root 24 days after inoculation with a nonterminal gall, showing nematodes in the fusiform condition typically oriented in the root tissues. Note darkened regions in the stele where giant cell formation is taking place. B. Pineapple terminal gall 27 days after inoculation, showing great increase in size of some of the female nematodes. Sections 1.5 or 2 mm. thick.

sumption of activity of the meristematic tissues. The result is a normal-appearing root tip growing out of the end of the old gall. This may continue to grow indefinitely as a normal root or it may be checked by another nematode infestation and produce another gall. Continued repetition ac-

counts for the beaded effect in roots, when galled regions alternate with normal root growth. Such a nonterminal gall is shown in figure 4, A, which is an illustration of a root removed 24 days after inoculation. Here are to be noted fusiform nematodes of fairly uniform size, arranged as described. in the cortex with their heads in the periphery of the stele. Attention is called to the region of development of the giant cells directly at the head of the nematode. The effect of the parasites in bringing about a very great enlargement of the central cylinder of the root shows strikingly in this photograph. Note, too, that the cortex is considerably thicker in this region of heavy infestation than in the normal part of the root. This latter appears to be a secondary effect, since there is no evidence of abnormal cell development in the cortex. It is the combined effect of the thickening of the central cylinder and the unusual thickening of the cortex that brings about the gall formation. Examination of the terminal gall shown in figure 4, B, discloses the very rapid increase in size of the female nematode, which clearly occurs sometime between the 24th and 27th days after inoculation. increase in size is shown to better advantage in figure 5, A, at 30 days, in which many nematodes have become flask-shape. The discoloration showing the region in the central cylinder in which giant cell formation is developing is also well shown in this picture. Figure 5, B, at 35 days, shows a number of fully developed females. The illustrations of development from the 24th to the 35th days show striking irregularities, which would seem to indicate that stage of development is not a function of time, alone, but probably of nutrition, as well. There is no question from examination of these pictures but that some of the females will mature and produce eggs very much in advance of others. It is to be regretted that none of the pictures shows males in the act of undergoing the final molt or of migration through the tissues. It is desirable to know the exact stage at which this takes place. This detail remains for further study.

In the series under observation, not until the 37th day (Fig. 5, C) was clear-cut egg-mass development discernible. This figure illustrates to excellent advantage the extruded gelatinous matrix in which the eggs are found. In two or three of the individuals shown in this picture the egg mass has already attained considerable size. It is to be noted that this egg mass is retained entirely within the cortex, that is to say, it in no place protrudes to the outside of the root as shown in the cowpea material, which was used for source of inoculation. That this has an important bearing on propagation of nematodes in the field will be pointed out in connection with the discussion of cowpea roots later in this paper.

When examining fresh materials of nematode galls it is virtually impossible to obtain a clear idea either of the number of nematodes contained therein or of stages of development of those nematodes. Prepared mate-

rials, such as were used for the illustrations shown here, have made such information readily available. Moreover, they should add considerably to the understanding of the processes of gall development.

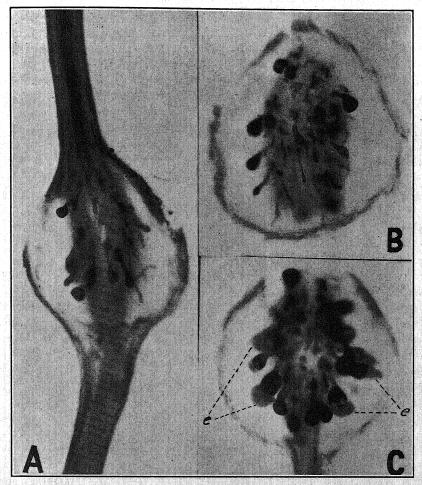


Fig. 5. A. Nonterminal pineapple gall 30 days after inoculation, showing flask-shape females. B. Terminal pineapple root gall 35 days after inoculation, showing fully developed adult female nematodes and many others not fully developed. C. Pineapple nonterminal gall, showing abundant adult nematodes with extruded egg masses (e). Regions of giant-cell formation are clearly shown here. Note also the abnormal thickness of the cortex.

OBSERVATIONS ON COWPEAS

As stated before, procedure with cowpea roots was essentially identical to that with pineapple roots. Because of the much more rapid root growth

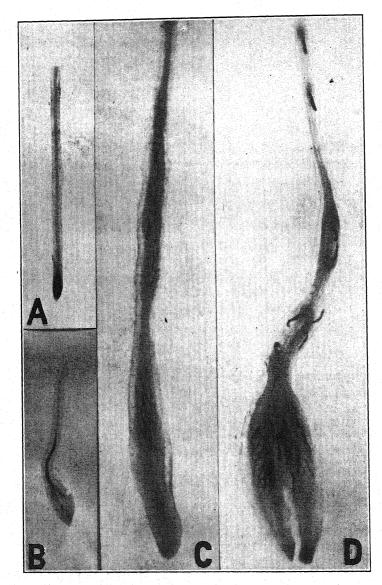


Fig. 6. Cowpea roots. A. 6 hours after inoculation. B. 48 hours after inoculation. Note that terminal swelling has already occurred. C. 4 days after inoculation, showing permanent orientation and beginning of growth of nematodes and distinct gall-tissue development. It is evident from the appearance of the root tip that it was still in active growth when it was cut from the plant. Due to this continuing growth, the nematodes are distributed over a greater length of the root than in pineapple roots of the same age. D. 7 days after inoculation, showing definite orientation of the nematodes in the tissues and distinct increase in size.

and branching in cowpea it was difficult to preserve the identity of originally inoculated root tips. In the later daily selection of more advanced stages of gall development the oldest appearing galls were selected in every case as the representative of the particular age desired. With greater abundance of tender cowpea roots available for infection, as well as more rapid root growth, it appears that there was greater wandering of the nematodes through the soil and, consequently, less concentration in any given root tip. The point of inoculation in the box was carefully marked, however, and it is believed that a fairly reliable range of root ages was selected.

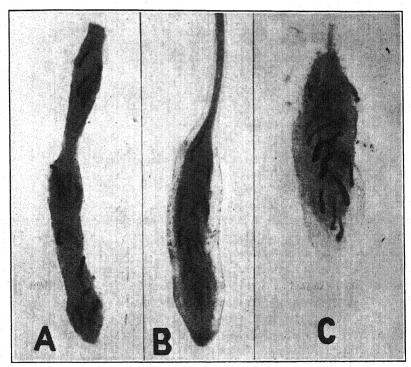


Fig. 7. A. Cowpea root 9 days after inoculation, showing nematodes in the fusiform stage. B. Infection may have been delayed here, for the nematodes do not appear to be so large as in A. This is a typical cowpea terminal gall. It shows enlargement of the stele to be primarily responsible for the root enlargement. C. Cowpea root 13 days after inoculation.

The early stages of penetration of nematodes in cowpea roots appear to be nearly identical with the same stages in pineapple. The first sign of gall development was perhaps manifest slightly earlier in cowpea than in the pineapple root. The cowpea root manifested the capacity to keep on growing, however, to a much greater extent than the pineapple. The result

was, apparently, the distribution of the nematodes within the tissues over a greater length of the root. This was due rather to continued growth of the root than to greater migration of the nematodes through the cowpea Figure 6, A and B, shows 6-hour and 48-hour stages, respectively. in a cowpea root. Figure 6. C, shows a cowpea root 4 days after inoculation and illustrates the distribution of the nematode through the length of the root. The appearance of the root tip indicates that it was still growing at the time the root was cut. Had the supply of larvae become exhausted in the vicinity of the root tip, the chances are that the root would have continued to grow and that gall formation would have appeared only in the particular region of the root shown in this picture, the root tip itself not being swollen. Already at this stage some of the nematodes had become fixed in the tissues and some growth had taken place. This, of course, means that migration has ceased, except, perhaps, for some larvae that may have entered the roots later than others. Figure 6, D. at 7 days, shows the relatively uniform orientation of the nematodes within the root tissues and the irregular gall formation in the root. Here most of the swelling appears to be in the central cylinder portion of the root, and relatively very little is manifest in the cortex. Distinct increase in size of the nematodes likewise is evident in this picture. In figure 7, A, 9 days, the nematodes are distinctly fusiform and are relatively uniform in size. We note at once that this stage is definitely comparable to the 24-day-old stage in pineapple. It is very evident, then, that development is taking place much more rapidly in the cowpea than in the pineapple. Later stages in the cowpea show it equally strikingly (Fig. 7, B). At 13 days considerable increase in thickness has occurred in some of the nematodes (Fig. 7, C) and at 15 days (Fig. 8, A) a sudden increase in size, manifest in pineapple at 27 days, has already occurred. Note in this picture the irregular outline that, in the fresh root, would appear merely as a slightly swollen, somewhat irregular root. Note, as well, the relationship of the nematodes to such swelling. This and the next picture (Fig. 8, B), 17 days, show particularly well the dark region in the central cylinder where giant cell formation is occurring. Figure 8, C, 19 days, shows the fully developed female nematode. It was in this slide, by careful focusing under higher magnification, that the first egg development was noted. The upper nematode in this picture shows an extruded gelatinous mass in the center of which 4 or 5 eggs were visible. Thus, under the conditions of this experiment, a new generation had commenced by the 19th day. This is to be compared with the pineapple plant growing under identical conditions in which eggs for the new generation were not produced until about 35 days after inoculation. This and the succeeding pictures show, as well, the extrusion of the egg mass to the exterior of the root. This is well shown in figure 9, A to D, at 21, 25, 26, and 29 days. Figure 9, C and D, shows it particularly well, for individual eggs are discernible in the egg masses.

Figure 9, E, at 30 days, shows a single relatively small gall in the cowpea root containing at least 5 nematodes and with protruding egg masses

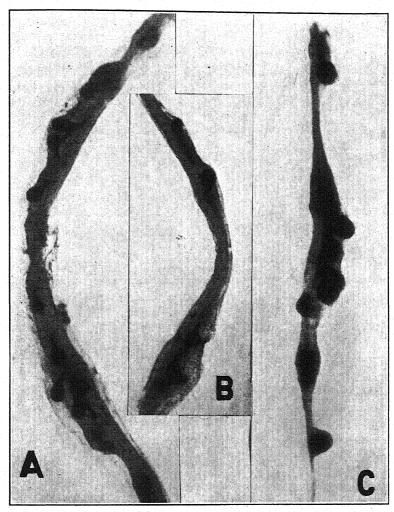


Fig. 8. A. Cowpea root 15 days after inoculation, showing very rapid increase in size of the nematodes after the thirteenth day. Note that the nematodes are completely covered by plant tissues. The stage of their development is not evident from a superficial examination of the fresh root. B. 17 days after inoculation. C. 19 days after inoculation. The upper nematode showed a small quantity of gelatinous secretion in which four or five eggs were to be observed.

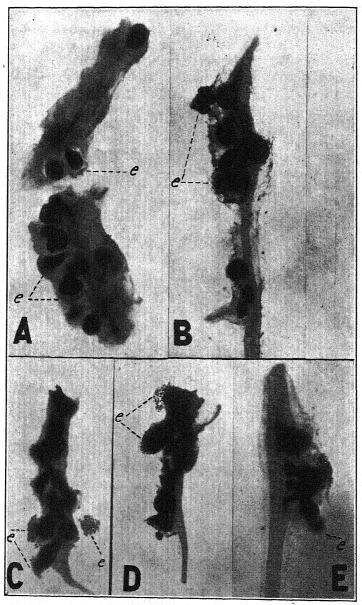


Fig. 9. Cowpea roots. A. 21 days after inoculation, showing several nematodes with extruded gelatinous matrix. B. Another root, 25 days after inoculation. Extruded egg masses are indicated by e. C. 26 days after inoculation. D. 29 days after inoculation. E. 30 days after inoculation. Egg masses with individual eggs evident are to be seen at e in C and D. C to E show egg masses protruding to the exterior of the root.

distinctly evident. This condition is comparable with figure 2 in the senior writer's paper on technique (12) taken directly from fresh material and showing the superficial egg masses. Figure 9, E, shows, as well, the region of giant cell formation in the stele.

Figure 10, A, at 43 days, is presented because it shows, in addition to the adult nematode and large protruded egg mass, the first infection by the new generation of nematodes in the region of root below the old gall. One would judge by the size of the nematodes that this infection is already 4 or 5 days old. The egg mass appears to be entirely empty, the larvae

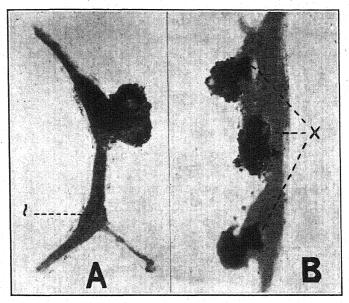


Fig. 10. Cowpea roots. A. 43 days after inoculation. B. 45 days after inoculation. Both show egg masses probably completely evacuated, the eggs having hatched and the larvae migrated. A shows a new infestation of second-generation larvae (1), apparently already 4 or 5 days old. B shows at X the collapsed bodies of dead females, their function in life completed.

probably having already hatched out and migrated away. Figure 10, B, at 45 days, shows the dead and collapsed body of the adult females and the empty egg masses attached to the side of the root. Adherent soil particles make the egg masses somewhat indistinct, but the appearance is typical of what actually occurs.

OBSERVATIONS ON EGG PRODUCTION

Egg production in nematodes within living cowpea roots, first observed on the 19th day, may be considered to have continued until about the 35th

day. Certainly, on the 30th day after inoculation it was still active, as may be seen by examining figure 9, E. On the 43rd day it may be seen from figure 10, A, that the maternal nematode has slightly collapsed and that the egg mass contains no new eggs; therefore, egg production must have ceased sometime prior to this date. Examination of other materials shows that it must have continued until at least the 35th day. This makes a period of about 16 days during which eggs have been produced. The length of this period, too, would seem to depend upon a factor of nutrition. Great variability has been observed between sizes of egg masses on different roots. Under highly favorable conditions egg masses have been observed that are considerably larger than the body of the female from which they came. It is to be presumed that for such heavy reproduction feeding conditions have continued favorable over a long period.

In an attempt to study the egg-producing stage in greater detail heavily infested cowpea roots, excised just before the egg-producing stage was reached, were used. Sections of such cowpea roots, each containing a single mature female nematode, were cut and placed on a white piece of blotting paper on a glass slide and this, in turn, placed in a moist Petri dish. Under these conditions the females continued their normal development and produced eggs for a considerable period. It was possible to make close observations on developmental stages by means of a binocular dissecting microscope.

Females were selected on the first appearance of the gelatinous mass, which was found to appear from 1 to 3 days before the first eggs were deposited in it. This external mass is closely attached to the body of the female in the region of the vulva. Within a day or two after its first appearance eggs are to be seen in the middle portion directly opposite the vulva. From this time on the gelatinous mass increases in size continuously as new eggs are produced and forced into it. The outer portion becomes hardened upon contact with its environment. Consequently, the earliest-laid eggs are confined well within the gelatinous matrix rather than pushed through it by the eggs deposited later. The egg mass adheres to the roots with some tenacity with the result that it becomes tightly packed. If the egg mass is dislodged it is observed that the latest eggs laid are not deeply embedded in the mass and may be separated at a touch.

Within a single egg mass may be found eggs in every stage of development, from the unsegmented forms to hatching larvae. A few eggs in the unsegmented stage were usually found in each egg mass, but eggs were most often deposited after they had reached the 2- or 4-cell stage. Eggs up to the 4-cell stage of development were frequently observed within the ovaries of egg-laying females. Similar observations are referred to by Atkinson

(1) and Stone and Smith (20). Nagakura (15), in his recent excellent paper on the embryology of *Heterodera radicicola*, does not mention it.

During the course of this study on egg deposition 7 females were under observation for a 10-day period. During this period the eggs deposited daily were removed from each female and counted. At the end of the 10-day period the bodies of the females were opalescent or nearly transparent, with the empty ovaries adhering to the body walls. In this condition their appearance contrasts sharply with that of females before eggs are deposited; at this time the body is completely white and opaque. A tabulation of the results of this observation is given in table 1.

TABLE 1.—Egg production from Heterodera females in excised roots during 10-day period

Female number	Days									A Tromo oro	
	1st	2nd	3rd	4th	5th	6th	7th	8th	9th	10th	Average
1	10 17 15 26 25 27 8	32 28 22 36 12 38 12	32 43 18 15 38 51 26	45 40 28 20 30 34 31	45 44 41 34 30 23 33	40 35 42 43 43 29 46	32 30 34 70 35 17 22	28 26 8 13 12 19 23	10 20 12 30 0 8 21	14 23 10 7 10 12 12	28.8 30.6 23.0 26.4 23.5 25.8 23.4
Average	18.3	25.7	31.9	32.6	35.7	39.7	34.3	18.4	14.4	12.6	25.9

It is to be recognized that these results are not comparable to what takes place in nature, inasmuch as the roots were not growing normally during the period of observation. Nevertheless, the fact that egg deposition varies from 23 to 30 per day in different individuals for the 10-day period, with a general average of 26 per day for the lot, is decidedly interesting. It is highly significant that mature females may continue to develop eggs for a considerable period, even though the root in which the female is embedded has been cut from the parent plant. This fact is of real importance in its relation to the use of the trap crop for nematode control.

Under normal conditions in the growing roots a female nematode may produce several times the number of eggs indicated by this table. During the progress of the present studies many egg masses were counted with between 600 and 800 eggs. As many as 1,200 eggs were counted from one mass, showing evidence of eggs that had already hatched and, likewise, evidence that new eggs were still being deposited at the time the egg mass was removed. Such large egg masses were to be found in both pineapple and cowpea roots. These figures are very much higher than those ordinarily given for numbers of eggs in *Heterodera radicicola*. The figures most commonly encountered in the literature are between 300 and 500 from Bessey

(3), Childs (5), Frandsen (7), and Newhall (17), 300 to 600 from Nagakura (15), while, from Africa, comes the low figure of 60, reported by Sandground (18). This difference is significant, of course, in that it makes evident the very rapid rate of reproduction under Hawaiian conditions.

DISCUSSION

The root-knot nematode is an obligate parasite, though it may remain alive for long periods in the soil in either the larval or the egg stage. It does not develop outside the roots of the host plant; consequently, the host is requisite for it to go through its life cycle. One of the most significant developments from this study has been the demonstration that, besides temperature, the host plant itself plays an important part in influencing the length of life history of the root-knot nematode. While the period required for penetration and the 1 or 2 days' migration period are approximately identical in cowpea and pineapple, the period required for development to the uniform fusoid form, common to males and females alike, is considerably longer in the pineapple than in the cowpea. The former required approximately 24 days for this, whereas, in cowpea, this stage was reached in only 8 or 9 days. The 27th day in pineapple is about the same as the 14th day in cowpea, showing that the time between the fusoid form and the beginning of rapid development of the female was approximately the same for both host plants. The first eggs were not observed until about the 35th day in pineapples, whereas they were seen on the 19th day in cowpea. This indicates again slower development in the later stages in the pineapple root than in the cowpea root. Table 2 is a condensed comparison of lengths of time required for developmental changes in the two host plants.

TABLE 2.—Comparison of length of life history of Heterodera radicicola in pineapple and cowpea

Gu	Time from inoculation				
Stage of development	Cowpea	Pineapple			
Penetration Migration ends	6 to 48 hours 1 to 3 days	6 to 48 hours 1 to 3 days			
Fusiform stage begins	9 days	24 days			
Rapid growth of female begins	13 ''	27 "			
First egg production	19 "	35 ''			

It is evident from this table that the greatest difference between the two plants lies in their influence on length of time required for the development from larval stage to fusiform stage. This may be a matter of nutrition. Possibly it is related to development to the fullest extent of the multi-

nucleate giant cells, the so-called nourishing cells (cellules nourricières) of Molliard (14) that appear to act very much like nectaries in providing a continuous supply of nutrient materials for the continued growth of the nematode. When once fully developed further growth and increased capacity for reproduction (volume of egg development) on the two plants do not differ materially.

These exact results on difference in length of life history corroborate very nicely the opinion of Bessey in 1911 (3) that "Time required for the development from the egg to the mature egg-laying individual depends to a great extent upon the temperature and upon the plant affected." Our several years' observations likewise verify Bessey's opinion with regard to the influence of temperature. The life history is very much longer in winter than in summer, even in Hawaii, where growing conditions prevail throughout the year. Miss Tyler's results (22) appear to be exact with regard to the relation of temperature to length of life history. In the light of our results, however, it is certain that her figures as to length of life history apply only to the particular host plant with which she was working. The minimum and maximum figures she has reported are 17 days at 27° C. and 63 days at 15½° C. Various other investigators have given length of life history in round numbers and without relation to temperature. During the period of our studies the air temperature of the greenhouse in which the observations were made ranged from 20° to 33° C. In all probability the soil temperature was near the optimum point for activity of the nematode the greater part of the time. As stated before, any absolute figures would require controlled temperature conditions.

The minimum length of life history might be considered to be the length of time that has elapsed from the time of inoculation to the hatching of the first eggs from the egg masses that developed as a result of this inoculation. Since, under the conditions of this experiment, the first eggs produced, 19 days after inoculation, required approximately 5 days to hatch, then, under these conditions, the length of life history was about 24 days. If these same conditions were to prevail throughout the year, then, with cowpea as the host plant, there might be said to be the potential possibility of 15 complete generations during the year. That this figure is far in excess of what actually occurs in Hawaii is evident from the known fact that life histories are very much slowed up in the winter months.

It should be noted that under ordinary field conditions nematodes are not to be found at any one season solely as eggs or as larvae or as developing organisms within roots of established host plants. Inasmuch as (1) length of time from larva to adult varies greatly within the same root, (2) eggs are produced during a period of approximately 2 weeks; and, finally, (3) they hatch during a period of several days immediately after, even the sec-

ond generation of nematodes is to be found in all stages in the field. Thus, the possibility of attacking nematodes at a season when one might expect an inherently weak stage in their life history, as is often done with insects, is eliminated from consideration in attempts at control, except by the trap crop method, before the first individuals have produced eggs. A pineapple root, typical of what actually occurs in the field, is shown in figure 11, A. Here, infections in the slow-growing root have apparently been continuous through at least 40 days, as nematodes in all stages of development are to be seen in the same root.

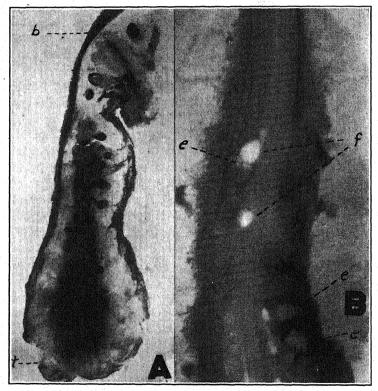


Fig. 11. A. Pineapple terminal gall taken directly from the field and processed by the usual method, showing all stages of development of the nematodes within the same root. In the newer terminal portion, t, larvae have only recently entered; in the older basal portion, b, are to be seen mature egg-laying females. About × 8. D. Longitudinal sction of an uncleared root from the field showing occasional female nematodes, f, and egg masses, e, entirely buried within the root tissues. About × 16.

Various early investigators, particularly Garman (9), Neal (16), Massee (13), and Frandsen (7) apparently have mistaken the dead body of the

female filled with developing eggs for the gelatinous egg-containing matrix. Likewise in this connection, Frank (8) says "... die befruchteten Weibchen zu birn—oder flaschen förmigen bis ½ mm groszen eiererfüllten Cysten anschwellen." Nagakura (15) makes clear the true nature of this body and refers to Voigt (23) (whose paper was not available to us) as having done so as early as 1891. At later stages this enclosing mass becomes thickened and brown and may be confusing because of its cyst-like nature. Indeed, this egg mass does function to some degree, as a cyst, in that it serves to protect the contained eggs and to hold them over for a considerable period during unfavorable conditions.

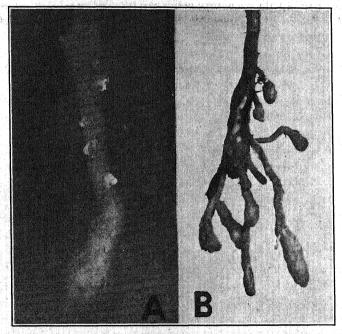


Fig. 12. Pineapple root knot. A. Case with protruding nematode egg masses. About $\times 4$. B. Much-branched root terminus, each branch with a typical terminal gall in which the egg masses are mostly borne internally, as in Figure 5, C. About natural size.

It is to be noted that the breaking through of egg masses to the exterior of the root is of considerable significance in the propagation of nematodes in the field. These eggs begin to hatch almost immediately under favorable conditions. Larvae are quickly released directly into the soil and are available for infesting such other roots as are in the immediate vicinity or as may be reached by the larvae in their migration under favorable conditions through the soil. This condition is to be contrasted with that shown in the

pineapple (Figs. 5, C, and 11) in which either slow migration of hatched larvae through the root tissues to the exterior or decomposition and breakdown of the root are necessary before the larvae reach the soil for infestation of new roots. In many cases this undoubtedly takes several months. It is a condition, of course, which provides protection of the nematode offspring through a longer period, but, at the same time, retards tremendously the propagation of new generations.

It is not to be concluded from this account that these two types of eggmass development are invariable with the two host plants. Actually, both types occur with both host plants. Figure 12, A, shows external egg-mass development in the pineapple as compared with the more typical condition of terminal-gall development, figures 12, B, and 11, with egg masses found in the interior.

Observations on time and manner of penetration indicate that, where there is a very large number of larvae present, penetration itself is likely to take place quickly, rather than otherwise, and to be complete so far as immediately available larvae are concerned. While there must be a first invader, as shown by figure 6, A, in cowpea, still, mass action appears to play a part in the heavy invasion immediately following. Figure 1, C, shows a large number of nematodes entering the roots at approximately the same point in preference to other available portions of the root-tip tissues. The resistance of surrounding soil particles, with consequent opportunity for the larvae to employ pressure, undoubtedly plays a part in their ultimate entrance.

SUMMARY

Cowpea and pineapple roots were inoculated by placing large numbers of *Heterodera radicicola* larvae at the tips of young actively growing roots. At 6, 12, 18, and 24 hours and at daily intervals thereafter, root tips were excised and immediately killed with Flemming's solution (which, by virtue of the contained osmic acid, stained the nematodes black) after which they were washed, gradually dehydrated through the alcohols, and finally cleared in clove oil. By this process the nematodes could be seen plainly in their natural positions in the root tissues in all stages of development from first penetration to maturity and the beginning of egg production. The root materials so preserved were used for making permanent slides and for photographic reproduction. The principal observations made thereon are recorded as follows:

1. The first larvae penetrate the root in less than 6 hours and penetration continues for at least 24 hours. Mass action appears to be a factor in heavy penetration of root tips. Large numbers of larvae often enter the root at or near the same point. Penetration is most abundant in the

meristematic region of the root tip beside the root cap, though entrance sometimes occurs through the root cap, itself.

- 2. Migration of the larvae occurs during a period of 2 or 3 days. This migration ultimates in locating a definite tissue in the maturing part of the root.
- 3. On or near the 4th day the larvae become definitely oriented within the root tissues. The head end becomes established in the cells of the endodermis of the cortex or in the outer periphery of the stele. The body lies in the cortex with the extremity lying diagonally towards the root tip. Thus oriented, they remain in this position, the females permanently, the males until the final molt at maturity.
- 4. Forward root growth is retarded within the first 24 hours after penetration. In cases of extremely heavy infestation, growth is permanently checked. In lighter infestation, after a period of retarded development, renewed growth, brought about by renewed activity of the meristematic tissues, often occurs. This is somewhat rare in pineapple root tissues but is the usual thing with cowpea.
- 5. The length of time elapsing from initial inoculation to the first egg development was, in cowpea, 19 days, in pineapple, 35 days. This difference appears to be inherent in the two host plants.
- 6. Egg masses in cowpea roots frequently break through to the exterior of the root. Here they are in such a position that the eggs often begin to hatch immediately, thus releasing a new generation of larvae into the soil for ready infestation of new roots. In pineapple roots egg masses are usually produced in the interior of the gall; thus release of the new generation of larvae into the soil is delayed and, consequently, the life history is still longer.

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INVESTIGATIONS ON APPLE BLOTCH1

EDWIN J. KOHL2

INTRODUCTION

Apple blotch, a disease of the cultivated apple (Malus Malus (L.) Britt.) and various species of native crab apples, is caused by the parasitic fungus Phyllosticta solitaria E. & E., which attacks the fruit, leaves, twigs, and buds of its hosts. It was first reported by Underwood (22), who collected it on the leaves of Pyrus coronaria L. near Crawfordsville, Indiana, in 1893. Underwood submitted this material to Ellis (5), who described and named the organism. Subsequently the disease has been reported from nearly every important apple-growing region east of the Rocky Mountains and south of 40° north latitude and has been made the subject of numerous investigations. Most of the extensive literature that has resulted is cited and reviewed in the publications of Scott and Rorer (20), Lewis (16), Roberts (19), Gardner, Greene, and Baker (9), and Guba (12). It, therefore, seems unnecessary to include a full review of the literature in the present paper. Such references to previous work as seem pertinent will be made in connection with the appropriate topics below.

Notwithstanding the excellent contributions that have been made by earlier workers, certain important gaps remain in our knowledge of blotch. Much difficulty has been encountered in tracing the development and maturation of spores in nature and no satisfactory technique has been reported for the consistent production of viable spores in culture. Infection experiments have consequently been seriously hindered, and many essential phases in the progress of disease improvement have remained obscure. In the present work, therefore, the attempt has been made to gain a more adequate understanding of the production of viable spores, both in nature and in culture, and of the phenomena of infection and disease development.

1 Joint contribution from the Department of Biology, Purdue University, and the Department of Botany, Purdue University Agricultural Experiment Station, La Fayette, Indiana. The results of these studies were presented to the faculty of the University of Wisconsin in partial fulfillment of the requirements for the degree of doctor of philosophy.

² The writer wishes to acknowledge his indebtedness to Dr. G. W. Keitt, University of Wisconsin, for suggestions and supervision in the prosecution of these studies; to Dr. M. W. Gardner, of the Purdue University Agricultural Experiment Station, for suggesting this problem and for active cooperation throughout the progress of the work; to Dr. H. E. Enders, Head of the Department of Biology, Purdue University, for much encouragement and stimulation throughout these studies; and to Prof. C. L. Burkholder, Department of Horticulture, Purdue University Agricultural Experiment Station, for field facilities and assistance.

SPORE PRODUCTION AND MATURATION IN CULTURE

The production of spores in artificial culture has long been known to be difficult of accomplishment. Scott and Rorer (20), Lewis (16), and Roberts (19) report production of spores on sterile apple twigs. Scott and Rorer state that sporulation was also brought about on corn-meal agar, but Roberts reports no sporulation on this medium. Guba (12) calls attention to the difficulties he experienced in obtaining fruiting cultures on any medium. He reports an instance of sporulation on corn-meal agar 7 days after transplanting the fungus but makes no statement as to whether the fungus was newly isolated in this instance. At another time, when sporulating cultures were obtained, he states that the age of the canker and the season of the year may have had some influence on fructification. He observed that subcultures never sporulated.

In the writer's studies, also, much difficulty was experienced in bringing the fungus to sporulation. A wide variety of artificial culture media was used. Corn-meal agar was used for the isolation of the fungus from fruits and twigs because of the ease of preparation of the medium and the possibility that the pycnidium-like bodies that are abundantly formed upon it might produce spores. The corn-meal agar contained 50 gm. of whole white corn meal and 20 to 25 gm. of agar per liter. The hydrogen-ion concentration of this medium was pH 5.3 to pH 5.5.

At various times it was observed by means of a binocular microscope that, in tube cultures produced by transfer of noncontaminated infected tissue from blotched Rome apple fruits, pycnidia were produced over the whole thallus. These pycnidia tended to round off and become somewhat spherical. When such cultures were incubated at 19° C. spores were found approximately 20 to 22 days after transfer. They did not germinate at once, a period of maturation seeming to be necessary. These results are similar to those of Guba (12), who notes that spores from culture sowed in water germinated only after some time had elapsed. After further trial it was found that, on this medium, the fungus in the original cultures isolated by tissue transfers from storage fruits of the Rome variety consistently produced fertile cultures (Fig. 1, A and B). Neither the use of whole yellow corn meal instead of whole white corn meal nor the use of tap water of 22 grains hardness instead of distilled water caused any apparent difference in results. New isolations were repeatedly made by this technique and the resulting cultures, which contained the transplanted host tissue, sporulated consistently.

Because lateness of the season prevented trial with other varieties and because Rome fruits presented clean smooth lesions, storage fruits of only this variety were used extensively. In earlier work cultures were made by this same technique from young apple fruits collected in late summer and

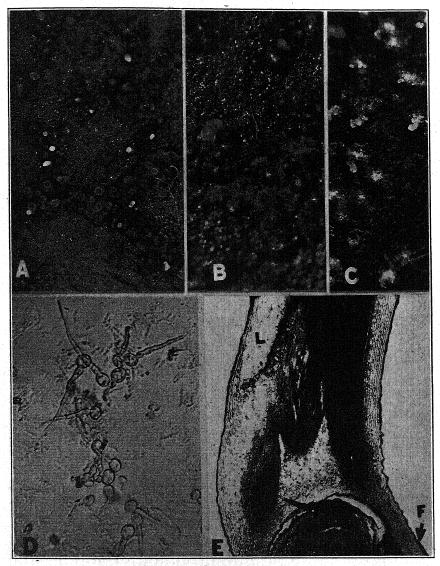


Fig. 1. A. A culture of the apple-blotch fungus on corn-meal agar, showing spores oozing from the fertile pycnidia. $\times 9$. B. Same as A. $\times 25$. C. Spore horns or cirri of the blotch fungus, extruded from the pycnidia on apple twigs, after being atomized in a moist chamber. $\times 15$. D. Photomicrograph of living blotch spores from natural sources, germinated in water on a glass slide, 18 hours after sowing. $\times 150$. E. Longitudinal section of petiole of apple, showing a blotch lesion on the left (L) and a bud. The mycelium of the fungus was traced into the abscission layer at F. $\times 25$.

early autumn as well as from diseased twigs, but no sporulation was observed. Much contamination occurred in cultures from diseased twigs.

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As was reported by Guba (12), subcultures obtained by making mycelial transfers failed to sporulate. No sporulation was obtained from transfer of spores or mycelium to sterile apple twigs in test-tubes. Similar subcultures on a variety of other media also failed to sporulate.

The data that have been presented strongly suggest that suitable living host tissues may influence sporulation. The exact nature of this influence has not been determined.

Guba (12) records spore production on artificial media at temperatures of 25° and 30° C. While no comprehensive studies regarding relations of temperature to spore production were undertaken by the writer, it was observed that certain cultures sporulated abundantly at 19° to 21° C., whereas parallel cultures at 28° C. failed to fructify. Temperatures of 19° to 21° C. seem to be very favorable for sporulation.

SPORE PRODUCTION AND MATURATION IN NATURE

Spore production and maturation in nature have been given some attention by Roberts (19), who reports a progressive maturation of blotch spores from natural sources, as determined by sowings made in water at daily intervals during May.

In the present work, germination studies were undertaken during several summers with spores from natural sources and from cultures. Many sowings were made in various dilutions from 0.1 per cent to 1 per cent of citric acid, hydrochloric acid, nitric acid, sodium oxalate, hydrogen peroxide, and boiled and fresh decoctions of apple fruits, apple leaves, apple bark, prune decoctions, and sugar solutions. Control sowings were made in sterile distilled water. In many instances similar tests were made with spores that had stood, without germinating, for 24 hours in drops of sterile distilled water. No instance was found in which the presence of any of the solutions used stimulated germination. Ether, chloroform, and carbon dioxide were liberated over sowings of spores in sterile distilled water, with negative results. When spore germination did result it was uniform within a small percentage for the spores contained in a single pycnidium. Because of the large percentage of spores that did not germinate during June, it is thought that not all pycnidia mature their spores simultaneously.

In order to ascertain at what time of the year spore formation and maturation occur under natural conditions another type of experiment was undertaken. Twigs with cankers, collected at Mitchell and La Fayette, Indiana, were brought into the laboratory at various times. Such twigs were placed upright in a moist chamber at approximately 22° C. and atomized daily with distilled water to cause the extrusion of spores. The

extruded spores had the form of spore horns or cirri (Fig. 1, C). The extrusion of spores was used as an approximate index to spore maturity. The results of these experiments, which appear in table 1, show that, from

TABLE 1.—A summary of results of experiments relative to morphological and physiological maturation of spores of Phyllosticta solitaria in twig cankers in 1927

Place and date of collection	Spores extruded after stated intervals in moist chamber	Germination of extruded spores on corn-meal agar			
Mitchell, Ind.					
January 20	3 weeks	None			
February 8	24 days				
'' 15	18 ''	원이 많은 살아보다 중심하다고 있었다.			
March 4	15 ''	신원하다 하는 경우 이렇게 되다			
" 10	9 "				
" 30	4 "				
April 1	3 "	12 days after sowing			
5	24 hours	18 " " "			
20	8 4	5 " " "			
" 27	5 "	5			
La Fayette, Ind.					
May 5	3	a a a a			
'' 15	3 "'	3 44 44 44			

twigs collected early in the season and placed in a moist chamber, spores were extruded only after a relatively long period of exposure to moist conditions. As the time of year approached when infection occurred under natural conditions, the interval between placing of the twigs in the moist chamber and extrusion of spores became progressively shorter. The interval between sowing and germination of the spores also shortened continuously. The extruded spores had every morphological indication of The lack of germination, however, indicates that they were not physiologically mature. No germination occurred in collections made before April 1. It was only after the spores had gone through a sufficient period of rest or afterripening that they germinated. The results of these studies would indicate that under field conditions as well as in culture there is an interval between the morphological and physiological maturity of spores. The period between the time of complete morphological maturity and physiological maturity was not successfully shortened by artificial means. In southern Indiana the spores, ordinarily, are morphologically mature by about April 1 and physiologically mature by May 1.

SPORE-GERMINATION STUDIES

Relations of moisture prior to germination. Considerable observational and experimental evidence suggests that the viability of spores may be

affected by the conditions of moisture to which they are subjected before germination. It is conceivable that the common lack of viability of spores in culture may be attributable in part to injury by drying, since it requires approximately 35 days for the spores to mature in tube cultures. Spores collected in the field after protracted dry periods and sowed on slides showed a much lower percentage of germination than those produced and naturally matured during moist weather. Evidence of injury to spores by desiccation was also obtained from field-infection studies that are herein reported. It is shown in figure 3 that, at La Fayette, Indiana, in 1925, a long period of drought followed petal fall. Infection did not occur after this period until 6 rains had fallen, the 3rd of which amounted to nearly an inch. sixth of these rains, during which infection occurred, was 0.6 inch. While such prolonged dry periods are infrequent, it is a matter of common observation and knowledge that during a dry season infection is relatively light, while during a wet season infection is quite heavy. Reference to figures 6, 7, 8 and 9 will illustrate how uniformly infection occurs during a continuously wet season.

Experiments were undertaken to determine the effect of drying on the germination of spores. During June and July spores were placed on bits of sterile cover glass and dried in the open air in the laboratory for periods of 1, 5, 10, and 30 minutes and 1, 2, 4, and 6 hours, respectively. None of the spores that were dried longer than 2 hours germinated on corn-meal agar. Spores that were left continuously in water as controls germinated vigorously within 12 to 24 hours. Numerous replications of this experiment gave similar results. These results, in conjunction with lack of natural infection when rains followed protracted dry periods, suggest that the viability of spores is sharply reduced by desiccation.

Adhesion of spores to substratum. The presence of some substance or envelope causing spores to adhere to their substratum was suggested by Voges (23) in germinating conidia and ascospores of Venturia inaequalis (Cke.) Wint. Keitt and Jones (15) made further studies of the phenomena of adhesion of the spores of the same fungus. They found that ascospores of Venturia exhibited a marked adhesion to the substratum within 15 minutes after they were sown in sterile distilled water on glass slides and that in 30 minutes 50 per cent of the spores were not washed off when the slide was held for 20 seconds in a strong stream of water falling from a faucet. Conidia of Venturia exhibited a similar marked adhesion. These investigators did not succeed in demonstrating the presence of an envelope of the type suggested by Voges.

Ordinarily, when mature spores of *Phyllosticta solitaria* are sown in distilled or tap water on physiologically clean glass slides, germination proceeds as has been described by Guba (12). It was observed in the

present writer's studies that at some time between sowing and the first appearance of a germ tube the spore becomes firmly adherent to the slide. This was demonstrated by placing the slide at an angle of 45° under a faucet attached to the city water system (pressure 50 to 70 lbs.) and allowing a stream of water of approximately 12 mm. diameter to fall upon it for 15 seconds. The germinated spores were still attached to the slide, whereas the spores that had not germinated were washed off. By inducing strong currents in open water mounts under microscopic examination it was clearly evident that both the spores and portions of their germ tubes were firmly anchored to the glass.

GERMINATION AND APPRESSORIUM FORMATION ON LEAVES

The germination of spores on leaves was undertaken with a view to determining the mode of entrance of the fungus into the host. By placing cankered twigs in a moist chamber and atomizing them with water, the extrusion of spores was effected within a few hours. Spores thus obtained were sown upon marked wet areas of detached leaves that were placed in a moist chamber. Germination was very sparse and erratic. Consequently, a different method was adopted. A small portion of a relatively clean canker bearing several pycnidia was cut away and soaked several minutes in water. It was then placed so that the ostiolar side of the pycnidia rested upon marked wet areas on the upper surfaces of relatively young clean Only distilled water was used in wetting the leaves. At intervals of 12 to 72 hours after inoculation the marked areas of the leaves were cut away, killed, and fixed for 24 hours in equal parts of 95 per cent alcohol and glacial acetic acid. They were then cleared in a saturated solution of chloral hydrate (Peace, 17), after which they were washed in water, stained in cotton blue or anilin blue, and mounted in glycerin. The material thus prepared was not suitable for imbedding in paraffin. When paraffin work was anticipated, formalin acetic-alcohol (Rawlins, 18) was used as a fixative and glycerin as a clearing agent, since glacial acetic-alcohol as a fixative followed by chloral hydrate as a clearing agent caused excessive hardening.

Spores that germinated on leaves differed from those that germinated on slides in that they formed appressoria. Appressoria have not been seen on slides. The germ tubes of spores sown in water on slides merely proliferated and branched profusely in a very short time (Fig. 1, D). On leaves, appressoria were formed in 20 to 30 hours (Fig. 2, A and B).

The term appressorium was used by Frank (6) in his studies of Fusicladium tremulae Fr. Appressoria have since been noted in a wide variety of fungi by many workers. They have been studied by de Bary (2) in Sclerotinia, Halsted (13) in species of Gleosporium, Aderhold (1) in Venturia inaequalis, and others. The writer has found no record of appressorium formation in other Phyllosticta species.

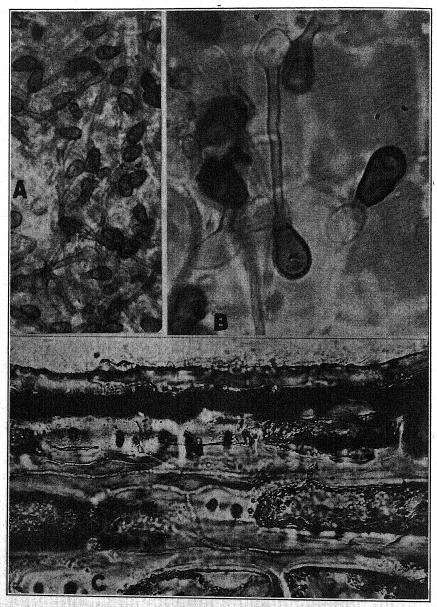


Fig. 2. A. Dark appressoria of spores of the apple-blotch fungus from natural sources, germinated on an apple leaf. $\times 200$. B. Same as A except that the photomicrograph was made with oil-immersion objective. Empty spores and germ pores of the dark appressoria are better resolved. Epidermal cells of the apple leaf are seen faintly below the appressoria. $\times 800$. C. Intercellular mycelium of *Phyllosticta solitaria* in collenchyma of the petiole, fixed in Schaffner's chromo-acetic fluid and nonstained. Septate hyphae may be seen. $\times 650$.

The appressoria of Phyllosticta solitaria are olivaceous brown. They usually are 10 μ long by 6 μ wide and are pear-shape to egg-shape in outline. Sometimes they are shaped much like a boxing glove. When first formed they contain a densely granular material. Later, a germ pore in the basal wall is quite evident and the content of the appressorium is less dense. Measurements and observations were made by means of a 1.40 N. A. oil-immersion objective. The germ tubes have the same olivaceous brown color as the appressoria and are from 4 to 14 μ long by 2 μ wide. Hyaline germ tubes have been observed on leaves and, in such cases, no appressoria were formed. It is of interest to note that spores germinated on glass slides form hyaline germ tubes and no appressoria. In all cases observed walls of the empty spores were hyaline and very difficult to see. It was only by the aid of an oil-immersion objective that they could be seen at all. In contrast, the dark germ tubes and appressoria were easily seen.

Mode of penetration. It was concluded by Blackman and Welsford (3) that the direct penetration of the cuticle of host plants by germ tubes or infection hyphae of parasitic fungi is a mechanical process and in no wise dependent upon local dissolution of the cuticle. This conclusion was made as a result of germination studies with Botrutis cinera Pers. They found that an appressorium of Botrytis can mechanically force a germ tube through a thin layer of gold leaf. Brown (4), however, states that a powerful enzyme is formed that can cause disintegration of the cell wall and the middle lamella and permit infection hyphae to penetrate the host cells. Gardner (7) found that Colletotrichum lagenarium (Pass.) Eriks. & Henn. also formed appressoria but that a distinct swelling of the cell wall was sometimes present at the point of contact between the appressorium and the cuticle, as seen in cross sections. In the present studies with Phyllosticta solitaria, the details of host penetration are not known beyond the formation of an appressorium. A germ pore can be seen in the basal wall of the appressorium, as the photomicrographs show. Attempts to reveal the details of penetration by means of paraffin sections have thus far been unsuccessful due chiefly to hardness of the material.

INOCULATION STUDIES

Successful inoculation and infection of apple trees with a suspension of spores of *Phyllosticta solitaria* from natural sources was reported by Scott and Rorer (20) and Roberts (19). Guba reports that his attempts to induce infection failed. The writer tried similar experiments by spraying young leaves and twigs with spore suspensions, but no infection resulted.

Further experiments were undertaken, therefore, to bring about infection. It was assumed that failure of earlier trials resulted from an excessive drying of spores. The method employed in these experiments is essen-

tially that previously described in this paper for the germination of spores on leaves. In a first attempt, small bits of cankers with pycnidia, previously soaked in water, were placed with ostiolar side in contact with the upper surface of the leaves and on petioles of potted apple trees. A piece of moist cotton was placed about each inoculated area and removed 24 hours later. In a second experiment, the inoculated twigs were enclosed in large test tubes plugged with moist cotton. In a third trial, in which moist chambers were used, as in the second experiment, spores from corn-mealagar cultures were substituted for the natural inoculum. Controls without the use of fungus inoculum developed no infection. In a fourth test potted disease-free Duchess apple trees, similar to those used above, were placed beneath an old diseased tree until a good rain had fallen, so that extruded spores carried in the drip from twigs might fall upon the potted trees. With each of the four methods, petiole lesions of the disease were produced. The symptoms were typical of naturally ocurring apple blotch. The fungus in each case was reisolated in pure culture by tissue transfer and showed cultural characteristics typical of Phyllosticta solitaria. Cultures of the reisolated fungus have not vet sporulated.

PATHOLOGICAL ANATOMY AND RELATION OF FUNGUS TO THE HOST TISSUES

The pathological anatomy of petiole and fruit lesions and cankers has recently been described by Guba (11). The present writer has made similar studies with results that have been generally confirmatory. Some exception to Guba's studies of the pathological anatomy of the canker, however, may be taken regarding the origin of the abnormal parenchymatous tissue beneath the phellem. He attributes this exclusively to the cambium, whereas the present writer has observed its origin from other tissues, notably the secondary phloem. Whether this abnormal tissue should be termed phelloderm is open to question.

Comparatively little attention has hitherto been paid to the progressive development of the fungus following host penetration and the relation of the fungus to the host tissues. Increasing understanding of the importance of petiole transmission, of which there has been much evidence from field and cultural studies of Gardner (8), emphasized the desirability of giving further attention to this phase of the subject.

Attention was given to methods of killing, fixing, and staining the fungus in order to permit a more rapid and satisfactory examination of histological sections. Material was killed and fixed in Gilson's fluid and stored in 80 per cent alcohol. Sections cut by the freezing method were placed in a 1–1000 gold chloride solution for about 30 minutes. The host cells took on a faint purplish color and the mycelium became granulated black. A fair degree of differential staining was obtained. This is the

method that was employed by Miss Gerry (10). The use of aniline stains or haematoxylins requires such heavy overstaining that the host tissue, in thick sections, obscures the mycelium. No staining at all is much preferable to such heavy overstaining for studying the mycelium in the host or tracing it across the abscission layer. It is notable that young mycelium stains with difficulty and older mycelium is very resistant to nearly all stains. Older mycelium can be traced rather easily because of its olivaceous brown color.

After the fungus has penetrated the host, it may be found in an intercellular position, as mentioned by Guba (11). In the petiole it limits itself to the collenchyma (Fig. 2, C). Although hundreds of sections were examined, the fungus was not found in any tissue deeper than the collenchyma. It could be traced a number of millimeters, longitudinally, in the collenchyma beyond the visible margin of the petiole lesion. Particular attention was given to tracing it on the side of the lesion toward the abscission layer. It was traced successfully, though with difficulty, through the abscission layer, so that we have microscopic evidence of petiole transmission of the fungus into the bark, where it may overwinter. Once in the bark, the fungus becomes established in the collenchyma.

Petiole transmission occurs during July or August, depending somewhat upon how early or how late infection has taken place. At any rate, in the vicinity of Knightstown, Indiana, as reported by Gardner (8), or La Fayette, Indiana, cankers infrequently appear in the autumn as the result of petiole transmission except where the lesion of the petiole was close to the bark and formed a small bark lesion directly. Such cases are rather uncommon. The mycelium in the bark is always in advance of the macroscopically visible edge or margin of the canker. The mycelium can be found any month in the year considerably in advance of the lateral edges of the cork tissue of which successive zones are formed in the cortex under the invaded collenchyma. The advancing mycelium can be traced readily through apparently normal tissue by the fact that the host cells show a yellowish or yellowish brown discoloration along the line of mycelial advance, except near the very tip of the most advanced hypha.

The relation of the fungus to the tissues of the fruit is of interest because of the extreme difficulty in locating the mycelium. This difficulty arises from the fact that the advancing hyphae are closely appressed to the walls of the host cells. A brownish discoloration of the host cells as a result of their reaction to the fungus almost completely obscures the fungus.

Petiole transmisson of the fungus has been definitely shown by Gardner (8) to be the principal means by which the fungus gains entrance into the bark of bearing wood. He found that a majority of the new cankers appearing in early spring occur at or near a leaf scar. By means of tags he demonstrated that such cankers appeared where a diseased petiole had

been attached the previous season. A similar case of petiole or leaf transmission of a fungus into the bark is that of *Cronartium ribicola* Dietr. on *Pinus strobus* L. as reported by Tubeuf (21). Hedgeock (14) finds also that, on Populus, petiole transmission is the means by which *Dothichiza populea* Sacc. & Briar. gains entrance to the bark for overwintering.

TIME OF INFECTION IN THE FIELD

For the study of the time of natural infection at La Fayette and Mitchell, 1-year-old Duchess apple trees were planted in 8-in. flower pots. These were sunk in rows some 400 feet from the old diseased trees. Either

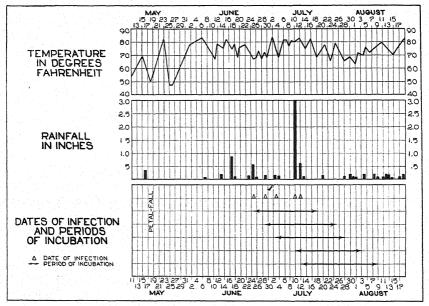


FIG. 3. A graphic summary of certain data relative to blotch-infection experiments and meteorological records at La Fayette, Ind., 1925.

2 or 4 potted trees were placed beneath a diseased tree, removed after the next rain, and replaced in the row of potted trees. During what was considered to be the blotch season, that is, from May to August, potted trees were exposed to infection in this manner throughout each rain. Records were kept of disease development in these trees. The meteorological records for the experiments at La Fayette were secured from the local weather station located at Purdue University and those for the experiments at Mitchell were obtained from the reports of the Paoli station, 12 miles distant. It is realized that the Paoli conditions varied slightly from those at Mitchell, but they constitute the best meteorological records that could feasibly be secured.

The times of natural infection for the years 1925 to 1928, inclusive, at La Fayette and for 1926 to 1928, inclusive, at Mitchell are shown in figures 3–9. Lesions produced on the petioles or midribs were used as an index to infection. Counts of the number of healthy and diseased leaves were made and records kept. The times of first and last occurrences of infection were recorded, and these have been projected in the graphs in figures 1–7. The experiments at Mitchell were at such a distance that the incubation periods could not be determined.

The summer of 1925 at La Fayette was relatively unfavorable to blotch infection (Fig. 3). Petal fall³ occurred May 19. Infection took place

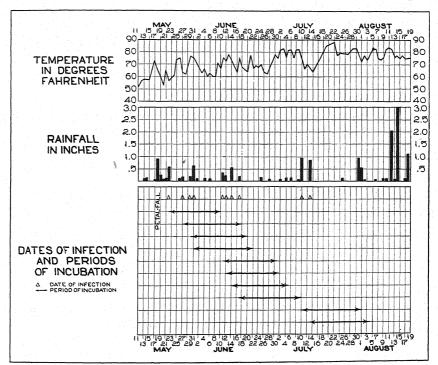


Fig. 4. A graphic summary of certain data relative to blotch-infection experiments and meteorological records at La Fayette, Ind., 1926.

during the 5 rain periods 36, 41, 45, 52, and 54 days, respectively, after petal fall. The protracted dry period early in the season undoubtedly delayed the first infection period considerably, as may be noted by comparison with subsequent seasons. Infection during all rain periods was light.

In the summer of 1926, at La Fayette, there were 10 rain periods during which infection took place (Fig. 4). Petal fall occurred May 19. Infection

³ The time of petal fall was recorded, when ½ to 3 of the petals had fallen.

took place during the rain periods, 3, 8, 11, 12, 23, 24, 26, 29, 52, and 55 days, respectively, after petal fall. First infection of this season almost coincides with petal fall, a fact noted also during later seasons, when environmental factors were predisposing to disease production. Data recorded during this summer show that a high humidity prevailed during spring and early summer and that infection occurred during nearly every rain. In late June and early July the humidity was quite low, even with light rains during alternate days. Under these conditions infection did not take place. Infection occurred incident to 1 in. of rain on July 10. There was no heavy infection on any of the potted trees.

The summer of 1926, at Mitchell, 150 miles south of La Fayette, was even less favorable to blotch than at La Fayette (Fig. 5). There were only

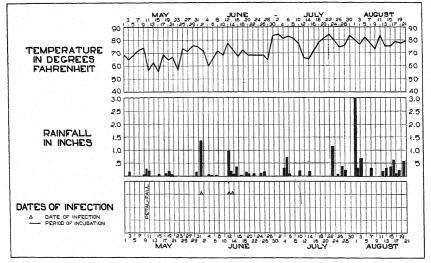


Fig. 5. A graphic summary of certain data relative to blotch-infection experiments and meteorological records at Mitchell, Ind., 1926.

3 rains during which infection occurred and infection was very light. These periods were 21, 32, and 33 days, respectively, after petal fall. Petal fall occurred May 11.

The summer of 1927, at La Fayette, was fairly favorable for blotch (Fig. 6). Rains were frequent, humidity was high, and consequently infection took place frequently. This was a rather early season. Petal fall was completed by May 7 and infection took place during 18 rain periods, 1, 3, 11, 12, 17, 18, 21, 23, 26, 27, 28, 36, 37, 42, 45, 68, 69, and 71 days, respectively, after petal fall. This represents a considerable number of infection periods. The effect of a dry period upon infection during late June may be readily seen inasmuch as no infection occurred during the

first 4 rains following the dry period. The heaviest infection occurred during the 2nd, 4th, 6th, 8th, and 12th rain periods, respectively.

A favorable blotch season was recorded also for the summer of 1927 at Mitchell (Fig. 7). Petal fall occurred April 25, and infection took place during 15 rains, 5, 6, 12, 13, 15, 19, 21, 24, 30, 31, 33, 35, 36, 50, and 58 days, respectively, after petal fall. The heaviest infection occurred during the 5th, 9th, 11th, and 13th rain periods, respectively.

Since the summer of 1928, at La Fayette, was exceedingly moist very heavy blotch infection took place (Fig. 8). Petal fall occurred May 13. There were 25 rain periods during which infection occurred. These occurred 3, 5, 6, 13, 17, 22, 24, 26, 31, 35, 36, 37, 38, 41, 42, 43, 44, 46, 47,

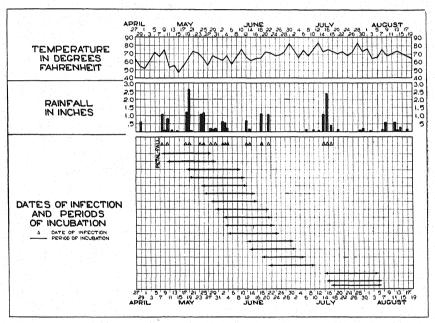


Fig. 6. A graphic summary of certain data relative to blotch-infection experiments and meteorological records at La Fayette, Ind., 1927.

51, 52, 53, 57, 61, and 67 days, respectively, after petal fall. Infection was heavy in all rain periods except the last 2 in which infection was relatively light.

Similarly, a very severe blotch season was encountered at Mitchell in 1928 (Fig. 9). Here petal fall occurred May 9. Infection took place during 22 rain periods, respectively, 5 days before and 8, 9, 11, 17, 18, 21, 26, 27, 28, 30, 31, 35, 36, 40, 42, 43, 46, 57, 61, 65, and 74 days after petal fall. This season, both at La Fayette and Mitchell, illustrates to a marked degree the relation of the abundance of rainfall to the frequency of infec-

tion. The infection that occurred before petal fall was a mere trace. During nearly all subsequent rain periods, infection was very heavy except during the last one. Infection at Mitchell was even heavier than at La Fayette during this season. As many as 43 leaves with petiole lesions were counted on 1 tree, representing 50 per cent of the total number of leaves present.

It may then be concluded that the time of first infection varies considerably, depending on the occurrence of early rains, which may coincide with the normal period when blotch spores are physiologically mature. Usually such a time of maturity immediately follows petal fall, although in one instance its advent preceded petal fall. An examination of figure 3 will show the decided effect of a protracted dry period on earliest infection.

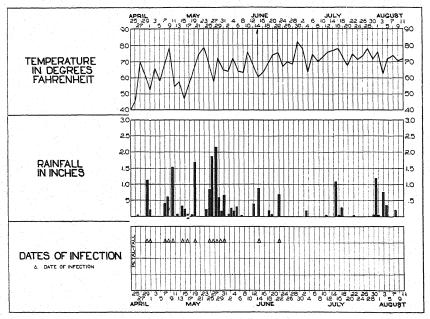


Fig. 7. A graphic summary of certain data relative to blotch-infection experiments and meteorological records at Mitchell, Ind., 1927.

The latest infection usually occurs during early July or the middle of July. Cessation of infection seems not to be dependent alone on lack of moisture but also on another factor. The supply of spores is by no means completely exhausted at this time, since spores continue to be formed in new cankers appearing during May or June. Examination of pycnidia in such cankers in late July and in August revealed abundant spores capable of germination. Since infection occurred no later than the middle of July, it may be assumed that the leaves had become resistant to penetration and infection.

From the data available there is no evidence that temperatures encountered within the usual range of field conditions during the period of natural infection have any sharply limiting influence upon infection. It may be that temperature shortens or lengthens the period of incubation, but no data are available to show that it does. The graphic records for La Fayette (Figs. 3–9) do not show any change in the length of the period of incubation attributable to differences in temperature. It is known from general observations that blotch epidemics are quite severe even during cool seasons. The summer of 1928 was rather cool, as well as moist, and infection was very heavy.

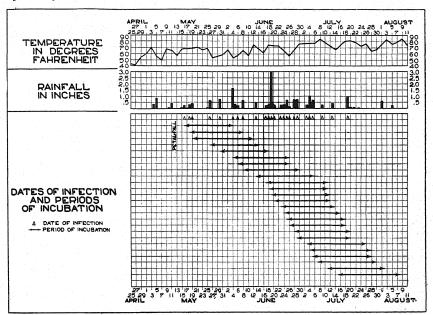


Fig. 8. A graphic summary of certain data relative to blotch-infection experiments and meteorological records at La Fayette, Ind., 1928.

It may be stated, also, that no definite correlation can be made between the occurrence or severity of infection and the amount of precipitation. Observations show, however, that during a period of high humidity a very light rain of approximately 1/10 in. or less may bring about infection, whereas a similar rain following a rather dry period may not be followed by infection. The time interval over which a rain falls undoubtedly plays a large part in bringing about infection.

PERIOD OF INCUBATION

The period of incubation varied from 18 to 24 days, as determined by greenhouse and field experiments at La Fayette. Because of the distance

to Mitchell, the period of incubation could not be determined there. The period of incubation under natural conditions and on trees infected artificially and kept in the greenhouse was about the same. These records are based on petiole lesions, although leaf-blade lesions were also present and usually appeared only a day or two earlier.

CYCLE OF INFECTION

In order to have a better understanding of the cycle of infection of the apple-blotch disease in nature, a large number of counts was made of infected leaves in an experimental block of 60 young Duchess apple trees at Vincennes, Indiana, in which cankers had been excised by M. W. Gardner

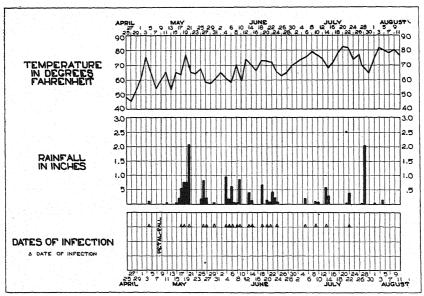


Fig. 9. A graphic summary of certain data relative to blotch-infection experiments and meteorological records at Mitchell, Ind., 1928.

since 1922 and blotch sprays withheld from 1925 to 1927. The counts of petiole infection were made in August, 1927, and the origin of the inoculum was determined by cones of infection that pointed to cankers at the apices. It was found that, in 37 trees that showed infection and in which 12,330 leaves were examined, no infection was traced to cankers on 1926 wood. Infection on 1,615 of these leaves was traced to 72 cankers on 1925 wood and only 2 cankers were found on 1925 wood under which there were no infected leaves. Infection on 35 of these leaves was traced to 6 cankers on 1924 wood. No infection was traced to cankers on 1926 wood nor on 1923 or 1921 wood of which 2 each were found. This is suggestive of a cycle

of infection at least 2 years in duration. However, in fruit counts made in 1928 in the same block of trees by M. W. Gardner, 6 instances of fruit infection were traced to cankers on 1927 wood, indicating an annual cycle of infection.

The field evidence indicates that with an early season the young cankers appearing in May, or possibly a few formed coextensively with petiole lesions the previous season, may be sufficiently well developed by July so that spores are mature and infection may occur. This is usually not the case in northern Indiana, but probably the severe epidemics in the Southern States may be explained by the fact that there is an annual cycle.

It may be seen, then, that in southern Indiana in seasons of moderate rainfall and infection, a 2-year cycle is completed, since spores produced in cankers appearing during the current season do not infect. During consecutive seasons that are moist and conducive to heavy infection and in which early infection occurs the first season and late infection the second, a condition is brought about whereby an annual cycle is effected. The fungus is able to produce spores in time to cause infection the second season; and protracted moist conditions cause a continued development of new leaves of the host, later in the season, that serve as an avenue for infection.

SUMMARY

- 1. Pycnospores were mature in overwintered cankers in late April at La Fayette and Mitchell, Indiana. In young cankers appearing in May and resulting from infection of the previous season spores were mature in July.
- 2. Fertile pycnidia were formed on corn-meal agar containing 50 gm. of corn-meal and 25 gm. of agar per liter in about 21 days on thalli containing the original tissue planting. Subcultures did not produce fertile pycnidia even on the same medium.
- 3. A favorable temperature for fructification was found in the range of 19° to 21° C.
- 4. A time interval elapsed between morphological and physiological maturity of the spores. Under natural conditions it was about 14 months.
- 5. Experiments designed to hasten maturation of spores through the use of various chemicals gave negative results.
- 6. Spores germinated in water on glass slides formed hyaline germ tubes only. The spores adhered to the slides and were not readily washed away by a stream of water from a faucet.
- 7. Spores germinated on leaves formed appressoria. Germ tubes and appressoria were olivaceous brown. Appressoria were, on the average, 6 μ wide and 10 μ long. Germ tubes averaged 2 μ wide and 4 to 14 μ long.

- 8. Young apple trees were successfully infected with spores from natural sources and with spores produced in artificial culture. The fungus was reisolated.
- 9. Spores produced after the middle of July or late July were of no great importance in the production of diseases at La Fayette and Mitchell, Indiana, probably because the host tissues were no longer susceptible.
- 10. By exposing in succession for suitable periods healthy potted Duchess apple trees to infection under a diseased tree, experiments during the seasons of 1925 to 1928, inclusive, have shown the time of natural infection to be from late April or early May to about the middle of July. Very early infection occurred when protracted wet weather coincided with the time of physiological maturity of the inoculum, which was commonly found to occur at about the time of petal fall. At LaFayette in 1925, infection occurred 36, 41, 45, 52, and 54 days, respectively, after petal fall. At La Fayette, in 1926, it occurred 3, 8, 11, 12, 23, 24, 26, 29, 52, and 55 days, respectively, after petal fall. At Mitchell, in 1926, it occurred 21, 32, and 33 days, respectively, after petal fall. At La Fayette, in 1927, it occurred 1, 3, 11, 12, 17, 18, 21, 23, 26, 27, 28, 36, 37, 42, 45, 68, 69, and 71 days, respectively, after petal fall. At Mitchell, in 1927, it occurred 5, 6, 12, 13, 15, 19, 21, 24, 30, 31, 33, 35, 36, 50, and 58 days, respectively, after petal fall. At La Fayette, in 1928, it occurred 3, 5, 6, 13, 17, 22, 24, 26, 31, 35, 36, 37, 38, 41, 42, 43, 44, 46, 47, 51, 52, 53, 57, 61, and 67 days, respectively, after petal fall. At Mitchell, in 1928, it occurred 8, 9, 11, 17, 18, 21, 26, 27, 28, 30, 31, 35, 36, 40, 42, 43, 46, 57, 61, 65, and 74 days, respectively, after petal fall.
- 11. Infection periods were found to be correlated with periods of rain and high humidity.
- 12. The incubation period is about 18 to 24 days, as determined by the potted-tree experiments.
- 13. The temperature range encountered in the experimental orchards during the season of natural infection was not found to cause any appreciable change in the length of the incubation period.
- 14. The fungus was found to be intercellular and, in the petiole and bark was limited to the collenchyma. This was demonstrated by the use of a 1–1000 gold chloride solution following fixation in Gilson's fluid. The host forms cork below the invaded areas. The mycelium in the collenchyma keeps in advance of the newly formed cork.
- 15. The fungus usually enters the bark of the host by way of the petiole, across the abscission layer, in late July and August.
- 16. In 1927, at Vincennes, Indiana, infection was traced to cankers found on 1925 wood. In 1928 a small percentage of infection was traced to cankers on 1927 wood. In 1927 the cycle of infection was found to be

at least 2 years in length. In 1928 the cycle of infection was found to be as short as 1 year in length.

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PEANUT WILT IN GEORGIA¹

J. H. MILLER AND H. W. HARVEY

This investigation was initiated in order to determine the identity of the organisms responsible for a very common wilt of peanuts in Georgia. It involved 3 disease surveys of about 1 week each, 1 beginning June 19, another August 8, and, the last, September 10. Besides the surveys, diseased specimens were sent the writers each week for study.

Most of the peanuts are grown in the southwest corner of the State on very sandy land. Nearly all the marketable peanuts are of the variety White Spanish, while those grown for hog grazing are Georgia or Alabama Runners. The large peanut, or Virginia type, on account of disease, has not been grown successfully.

Last year experiments were conducted in various counties with the Virginia type, with very poor results. Gypsum was used as a dust, as is done in Virginia, but with no evident control. Climatic conditions, being very dry in the early summer and wet in the late season and during harvesting, were very unfavorable. At Ft. Gaines, Virginia Runners yielded 1,036 lbs. per acre; Virginia Bunch, 816 lbs., and White Spanish, 1,305 lbs. This was on sandy land and there was much Sclerotium rolfsii Sacc. in the late season. At Cuthbert 1,818 lbs. per acre of Virginia Bunch was harvested. This tract is a red clay, and there was much less disease than on the sandy types of soil.

This summer, conditions were not especially favorable for growth of fungi, but there were more wilt and root rot than last year. Where peanuts had been grown for successive years on the same land the loss was heavy, and it was also considerable on some fields that had not been planted to this crop for 3 or 4 years.

METHODS

Roots or infected areas or stems of diseased plants were washed thoroughly in distilled water, then soaked in mercuric chloride for 5 minutes and placed in Petri dishes in hard agar. Most aerial molds were eliminated in this manner. Pure cultures were then made of all probable pathogens, and these were used to inoculate peanut plants grown in the greenhouse. Supplemental inoculations were made in the field. Positive

¹ The writers wish to express their appreciation to the Tom Huston Peanut Co. for its cooperation in this investigation and especially for the aid rendered by Mr. Bob Barry, Mr. Grady Porter, and the county agents in this district. The disease surveys were accomplished in cooperation with the Division of Mycology and Disease Survey, Bureau of Plant Industry, United States Department of Agriculture.

results were obtained with 3 organisms isolated from a majority of the diseased specimens. These are described below.

1. FUSARIUM MARTII APPEL AND WR. VAR. PHASEOLI BURK.

This organism produces a peanut root rot, which results in the affected plant's first turning yellow, then wilting, followed soon by death. The most severe infections were found in many fields in the region about Blakely and Ft. Gaines. During the June inspection, in one field near Blakely, from 20 to 38 per cent of the plants were either dead or dying. At the Huston Experiment Farm, Ft. Gaines, about 20 per cent of the plants wilted during June. Cotton had preceded peanuts on most farms and no information could be obtained as to root rot or wilt of cotton or other crops planted here in other years.

Hosts. The peanut varieties found to be susceptible were White Spanish, Virginia Runner, Virginia Bunch, Georgia Runner, Alabama Runner, and Namberquarie. The White Spanish was most susceptible, followed by the Virginia forms, and the Namberquarie least, with Alabama Runners fairly resistant. Burkholder (2) describes this variety as causing a dry root rot of several different kinds of beans of the genus Phaseolus, also of black-eye cowpea and of kulti bean. The writers performed no experiments to determine the susceptibility of plants other than the peanut. No previous record has been found of its attacking the peanut.

Description of the disease. The maximum period of infection occurs when the plants are about 2 months old and just blooming. In some cases only 1 or 2 shoots wilt, but in most instances the entire plant succumbs. Because of the drought at this period, there were very few lateral roots or adventitious roots or peduncles in the ground; so the entire water supply came through the tap root. On examining the roots, necrotic areas were found in the upper part, just below the crown (Fig. 1, A). The root at this point was completely rotted, became brittle, and broke off very easily.

The first signs of the disease consist in a yellowing of the upper leaves, and during the drought this condition was followed quickly by wilting and death of the plant. There are no external signs to suggest a Fusarium as the causal agent. Determinations can be made only by laboratory study.

Very young lesions begin as small, elongate spots, slightly sunken, with light centers and dark brown borders. As the lesion ages, the root is girdled, the spot increases to 1 to 2 cm. in length, the epidermis and cortical tissue become shredded, and the root dies. Cross and longitudinal sections were made through the necrotic area. In the disintegrated cortical region and also in the xylem, fine hyphae were found. The epidermis, cortex, phloem, cambium, and xylem rays are completely destroyed. The walls of xylem vessels are darkened. These anatomical changes extend up for 2 or more

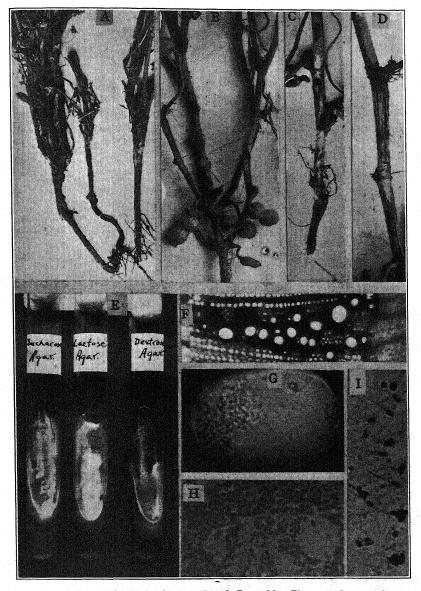


Fig. 1. A. White Spanish plant gathered June 26. The rotted roots in culture produced the organism Fusarium martii var. phaseoli. B. White Spanish, collected August 10, Huston Experiment Station, showing bacterial lesions on lower parts of shanks. C. The same showing lesions higher up on stems. D. Bacterial lesions enlarged showing characteristic shredded appearance when old. E. Agar slants of Bacterium solanacearum, 8 days old. F. Cross-section of stem (×195), showing tracheal tubes plugged with bacteria. This is to be seen in the very black areas. G. A single vessel showing bacteria. ×1090. H. Bacteria from pure culture. I. Bacteria showing polar flagella.

centimeters beyond the visible lesion. There is no plugging of xylem vessels with hyphae, as Atkinson (1, Fig. 6) described for Fusarium vas-infectum in cotton roots.

Wilted plants were observed scattered all over the fields, with no appearance of central focal points. There was not much evidence to make one suspect that the fungus was spreading from one plant to another.

Etiology. The pathogen, Fusarium martii var. phaseoli was determined as such by Sherbakoff. In litt. Aug. 8, 1931, Sherbakoff states that he considers Burkholder's organism to be identical morphologically with his F. martii var. minus Sherb. He further says that the variety phaseoli morphologically is about the same as the species martii but differs from it in causing a dry root rot of beans.

Although Burkholder (2, p. 1,024, tab. 3) has proved the causal relation of his organism with root rot of bean and while this organism resembles it morphologically, no experiments have been made to prove them physiologically identical.

Inoculation experiments. Sixty plants were grown in pots in the laboratory. Selected seeds were hulled, then soaked for 5 minutes in mercuric chloride (1:1000), washed in distilled water, and planted in pots containing sandy loam. The soil and pots were sterilized for 1 hour at 20 lbs. pressure. After these plants had grown about 6 in. high 20 were inoculated with the Fusarium conidia by puncturing the epidermis just below the crown and 20 were inoculated by pouring a spore suspension on the soil. In 10 of the latter some roots were broken, and in 10 they were not broken. Twenty pots were left as controls. In all cases where abrasions had been made on roots the plants wilted within 5 days, but negative results were obtained where the epidermis was not first ruptured. The wilted plants were examined, and the Fusarium was found growing on decayed roots.

An inoculation experiment was then made to determine the toxicity of a Fusarium filtrate. Ten flasks containing Richard's solution made up according to Fahmy (3, p. 545), differing from the standard in containing 1,500 cc. of water instead of 1,000 cc., were inoculated with the fungus, and a copious growth was obtained within 15 days. Then each was filtered under sterile conditions, and young plants were placed in 6 flasks along with parts of mature plants. These wilted within 3 days (Fig. 2, A). Similar plants placed in the solution without the fungus did not wilt at end of 15 days.

Field inoculations on mature plants were made on August 10 at Ft. Gaines. These plants were examined September 10 and no wilt was produced. The Fusarium was reisolated from a few decayed roots and peduncles but no serious disease was produced on these mature plants.

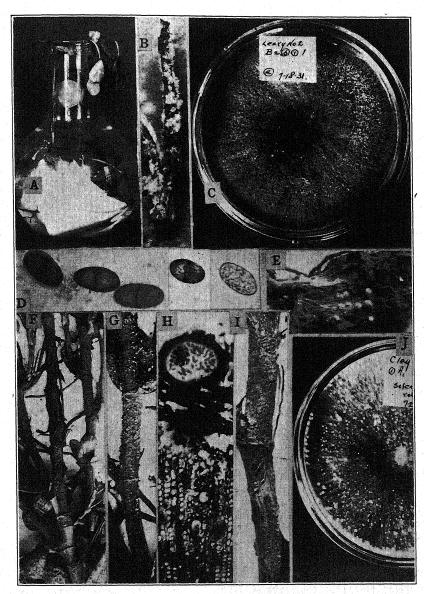


Fig. 2. A. Wilted plant after 48 hours in filtrate from Fusarium martii var. phaseoli, growing on Richard's solution. B. Fusarium developing on affected crown and root in dextrose bean agar. C. Pure culture of Fusarium martii var. phaseoli, 40 days old. D. Conidia of Diplodia natalensis. ×655. E. Sclerotia on roots and soil. ×9. F. White Spanish plant showing Diplodia pycnidia covering lower branches. G. Part of F enlarged by 10. H. Cross section of plant showing Diplodia pycnidium and disrupted cortex, phloem, and cambium. ×195. I. Lower part of stem, ×9, of White Spanish, August 10, showing mycelium of Sclerotium rolfsii. J. Pure culture of Sclerotium rolfsii from Huston Experiment Station.

Description of fungus. The following characters were brought out by growing this fungus on the various media described below.

Micronconida on aerial mycelium typically present, mostly O-septate, $2.8-14\times3.5-5.6~\mu,$ 1-septate $14-22.4\times4.2-5.6~\mu,$ oval to oblong, occasionally 1-septate spores constricted at septum; macroconidia mostly 3-septate, of nearly uniform diameter throughout, usually slightly broader toward base, rounded apices or slightly constricted, $24.4-39.2\times4.2-6.3~\mu.$ Aerial mycelium white drab; substrate on neutral agars brown to reddish brown or reddish orange on rice; chlamydospores single or in chains, verrucose when mature, $8-12~\mu$ in diameter, globose, intercalary or terminal; conidia mostly borne in false balls at apex of long conidiophores (Figs. 2, A–C and 3).

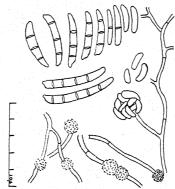


Fig. 3. Fusarium martii var. phaseoli showing micro- and macrospores and chlamydospore formation.

Cultural characters. Parts of roots with lesions were cut off and sterilized for 5 minutes in mercuric chloride (1:1000) and plated in Petri dishes in hard, bean agar. This was done with all specimens collected in the June inspection and, from then on, from plants sent in from this region. The Fusarium appeared on 80 per cent of such lesions within from 2 to 3 days. It grew very slowly on such stems, producing small sporodochia, such as are seen in figure 2, B. These were grayish white in color. After 4 or 5 days these cultures were overrun with aerial molds. Pure cultures were obtained by lifting off single spores, or a false ball of spores, from a single conidiophore and placing them on sterile media.

Much aerial mycelium with 85 to 90 per cent of microspores was obtained on rice and peanut agar and on whole wheat grains and broken steamed peanuts. Chlamydospores appeared on the rice and peanut agar but not on the other 2 media. The color change was most pronounced on the rice agar.

Very little aerial mycelium was produced on potato-dextrose agar, wheat agar, bean-sucrose agar, and plain potato agar. The spore types on these

media were 80-90 per cent 3-septate macrospores with a very small percentage of 4- and 5-septate spores and very few microspores. Small pseudopionnotes of macrospores formed on all of these. Also, few chlamydospores developed.

On sterile agar very few conidia formed from scant aerial mycelium, but chlamydospores developed abundantly.

Effect of weather conditions and age of plant. This fungus produces most serious damage to young plants during hot dry weather. These facts are shown by the inoculation experiments in which very young plants in pots wilted within a few days; and, although mature ones in the field 10 days after inoculation showed some evidence of root rot, it was not sufficient to damage seriously the production of peanuts. In the field the effect was worse during the extremely hot days of June and early July, when the plants were young and the root systems poorly developed. After the rains began in middle July the damage from Fusarium was negligible. Burkholder (2, p. 1021) says: "The effect of the fungus is rather to kill and dry up the tissue as it progresses up the root, thus greatly reducing the root system of the plant. The roots that remain healthy or are able to carry on their normal function are the surface roots." "If a prolonged dry spell occurs at the time of blossoming and pod production, it is very injurious to the diseased plants. The yield, without doubt, will be reduced over fifty per cent." These statements apply very well to the peanut wilt.

2. BACTERIUM SOLANACEARUM E. F. SM.

Most of the plants affected with dry root rot also contained bacterial lesions on the lower parts of stems. As the summer advanced less Fusarium was observed, and, beginning with the middle of July, the majority of diseased peanut plants contained this organism.

The distribution of this bacterial wilt in Georgia was more wide-spread than that noted for *Fusarium martii* var. *phaseoli*. At first it was seen chiefly in Clay and Early counties, but later in the season specimens from all the peanut-growing counties exhibited this disease.

A wilt caused by this organism has been noted before. Van Breda de Haan (5, p. 151) describes from Java a type of wilt that seems to be identical with that found in Georgia. The xylem vessels are blocked with bacteria and the plants turn yellow and die. Fulton and Winston (4, p. 43) report a similar condition in North Carolina.

Hosts. The varieties White Spanish, Virginia Bunch, Virginia Runner, Alabama Runner, Georgia Runner, and Nambyquarie were all infected, but the White Spanish appeared to be most seriously damaged and the Nambyquarie least. All of the above Runner types were attacked but with much less wilt than the Bunch types.

Besides the peanut many other plants are susceptible. Stevens (8, p. 36) mentions Datura, *Solanum nigrum*, Physalis, Petunia, Nasturtium, bean, pea, Ricinus, vanilla, Helianthus, Dahlia, Cosmos, tomato, tobacco, and potato. As the cause of the Granville wilt of tobacco it is very common in parts of North Carolina and occurs occasionally in Georgia.

Hartley (6) reports that tests were conducted in Java to determine susceptibility of various varieties of the peanut to *Bacterium solanacearum* and that American varieties, especially the Valencia, proved more susceptible than the Javanese varieties at Buitenzorg. The Tjina variety was most resistant. All plants on contaminated soils became infected.

Description of the disease. The term "wilt" does not apply to all the symptoms exhibited by plants affected by this bacterium. Only young plants and an occasional branch on a mature plant actually wilt. The term "yellows" is more expressive of the symptoms shown by mature plants.

The diseased plants at first turn yellow, and, if the attack be severe, this condition is followed by death of the entire plant or of single branches. When the single branch died death was found traceable to bacterial lesions on the stem, such as is seen in figure 1, B, C, and D.

The field signs of the disease are to be seen in the wilting of leaves and stems after they have turned yellow. The bacterial wilting can be determined by the dark brown spots, visible to the eye in cross-section, in the xylem and pith. This character serves to distinguish it from the root rot caused by Fusarium. The latter does not enter the xylem appreciably except in extreme cases of rotted roots.

The lesions begin as small black spots, which spread rapidly in a radial direction. A large lesion (Fig. 1, D) shows a light slightly sunken area surrounded by a dark margin. The central part is composed of shredded epidermis and cortical tissues. This splitting is typical of an old bacterial infection.

Histological changes. Infected parts were fixed and killed in Flemming's Weak, Flemming's Strong, and Carnoy Solutions, embedded in paraffin and sectioned. The young plants cut very well, but older ones containing much lignified tissue have to be sectioned from celloidon. Sections were cut rather thick (10, 15 and 20 μ) in order to hold the bacteria. These were stained with anilin-fuchsin and iron-alum-haematoxylin, counterstained with safranin. The latter combination gave the best results.

The bacteria penetrate either through an insect wound or directly through lenticels, spreading laterally as well as towards the center. Epidermis, cortex, phloem, and cambium are killed and cell walls become stained brown. Xylem vessels become packed with bacteria, and living xylem rays are destroyed. (Fig. 1, F.) In this stem cross section the

darkened areas surrounding empty vessels are cells that have become entirely occluded with bacteria. In figure 1, G is seen a single vessel in cross-section showing bacteria. In the pith brown areas are filled with bacteria.

Etiology. This organism has been identified as a causal pathogen of peanut wilt by van Breda de Haan (5) and by Fulton and Winston (4). Proof of this is shown again by the following experiments.

Inoculation experiments. Several hundred plants were grown in sterile soil from seed treated as explained before. When the plants were about 6 in. high they were divided into 3 lots; 1 was inoculated by rupturing the surface with a needle containing active bacteria, another by pouring a suppension of bacteria in distilled water on the soil, and the last lot was kept for a check.

At the end of 8 days all plants appeared to be approximately even, though the inoculated ones were slightly yellowed. Some of these were pulled up and examined. Where the soil had been inoculated the tap roots were black and shrivelled and almost dead, but many surface roots appeared to be keeping the plants alive. Cross-sections above the darkened areas showed bacteria in pith and xylem, with characteristic browning, even above the crown. In the stem-inoculated plants the lesions did not increase much in size, but bacteria were found in xylem and pith. Most of these died within 3 weeks.

Field inoculations were made on mature plants at Ft. Gaines and at Athens on August 10 and 21, respectively. Stems were inoculated, and dilutions were poured on the soil at the bases. Examinations were made on August 29, with no macroscopic results, but such stems, when sectioned, showed bacteria in xylem vessels. On September 10 all inoculated plants had become distinctly yellow in contrast to the green of the noninoculated ones.

Effect of weather and age of plant on bacterial growth. High temperatures are more favorable to the growth of the organism, and this accounts for the wide-spread prevalence in the Southern States and its nonoccurrence very far north. It grows more rapidly in young plants during very hot weather. The attack was greatest in June and July. For successful inoculations the writers found that bacteria had to be taken from 2- to 3-day-old cultures and placed in young plants. Such plants, very succulent, were more conducive to rapid growth and spread of the organism. In mature plants bacteria filled up some of the vessels in the xylem and appeared to become quiescent quickly, and not enough of such vessels were occluded to cause death. However, occlusion produced a slight yellowing of leaves, and such plants were more rapidly attacked by such partial parasites as Diplodia natalensis Evans.

Control. At the Huston Experiment Farm both sulphur and copper sulphate were used in a control experiment. These fungicides were placed in the ground when the peanuts were planted and were also dusted on plants about a month old. Only negative results were obtained.

This is definitely a soil organism, and, so far, the only means of control advanced are to be found in a long rotation. The important point here is to know what plants are susceptible, including weeds, and to keep these off the ground for a period of at least 5 years, according to Stevens (9, pp. 26 and 257). Wolf and Stanford (11, p. 164) add to this list many weeds common in cotton fields, so that clean cultivation should be practiced with a resistant crop.

No work has been done in breeding resistant varieties of peanuts in this country, but such has been done in Java, and it is possible that resistant Javanese varieties could be established here.

3. DIPLODIA NATALENSIS EVANS

This fungus was found in the late summer in fields that had been severely infected with bacterial wilt. It appeared to be causing a foot rot on from 10 to 20 per cent of plants in 1 field in Clay County. Many plants, still green and healthy in the tops, were covered with pycnidia at the base.

Cultures were made from single spores obtained from pycnidia on peanut stems. Inoculations were then made from the resulting mycelium and also from spores on plants grown under control conditions and on many plants in the field. These attempts all uniformly failed to produce infection.

All plants containing the Diplodia in the field also had *Bacterium* solanacearum in the vascular system as well as bacterial lesions on the stems. The evidence seems to justify one in concluding that this species of Diplodia is a weak parasite following attacks of more virulent organisms.

The position of the pycnidium in the decayed cortical tissue is shown in figure 2, F, G, and H.

4. SCLEROTIUM ROLFSII SACC.

This organism is wide-spread over the entire peanut belt, having been isolated from specimens from almost every county. There has been no special epidemic in Georgia, but it was particularly severe in isolated fields. The greatest losses were in light sandy lands near Ft. Gaines. In one 60-acre field the yield was reduced to about 600 lbs. per acre, which is a 50 per cent decrease of an estimated yield in July. At the Huston farm last year the yield of White Spanish was 1,305 lbs. per acre with much Sclerotium rolfsii, and this year, on the same land, that yield was cut to about 300 lbs. per acre, due chiefly to this organism and the bacterial wilt.

This fungus attacks living plants, and, from data gathered the last 2 years, it is worse during the late part of the season, especially if there is much rain.

During the month of August about 25 to 75 per cent of plants sent in from Clay County had this infection. Because of a very dry fall not many plants died, but the injury to roots and peanuts was considerable.

This disease is not a new one on peanuts in the Southern States, although it appears to have been overlooked as an important one in Georgia. Wolf (10, p. 143) says: "This disease appears to confine its attacks to the roots and peas. Usually there is no indication of the disease in the appearance of the aboveground parts. When, however, the plants are dug a greater or less proportion of the peas will be found to have decayed." Besides this citation from Alabama, McClintock (7, p. 441) found it causing a wilt in the peanut plots of the Virginia Agricultural Experiment Station.

Hosts. This organism is known to produce wilt, blight, or root rot on a great many hosts. Wolf (10, p. 147) found many legumes susceptible, but the Brabham and Iron cowpea escaped attack. McClintock (7, p. 448) says: "Tests of six varieties of peanuts and one hog goober, Worandzia subterranea, planted in soil badly infested with S. Rolfsii showed that the Valencia peanut is most susceptible to this organism and that Spanish, Tennessee Red, and Virginia Bunch are respectively resistant in the order named, while the Virginia Runner, African, and Hob Goober are practically immune to S. Rolfsii."

Description of the disease. This summer the writers found that most of the trouble would come under the heads of root rot and rot of lower shanks and a great deal of rot of peanuts rather than wilt. There was very little actual wilting of entire plants. In most cases only single branches died, and when such plants were pulled up the fungus was found covering roots and peanuts. During the short rainy season in midsummer affected plants contained a thick envelope of white mycelium on lower shanks with many developed sclerotia, but in the following drought the fungus was confined to parts below the ground and the growth of mycelium was much reduced. All parts covered with this mycelium turn brown and decay rapidly.

No attempts were made to prove the parasitism of this organism, as that has been done by McClintock (7) and Wolf (10).

Control. There are no known methods of soil treatment that successfully control this disease in the field. Experiments with soil disinfectants at Ft. Gaines were unsuccessful. Also, the great number of susceptible plants makes it impracticable to attempt to eradicate it by crop rotation. The only hope lies in planting resistant varieties of peanut in sandy lands

known to be infested. This fungus rarely becomes a serious problem on heavy land.

The writers have found the Alabama Runners least susceptible of the varieties grown in Georgia. Virginia Runners are also fairly resistant, but the pods are so large and they mature so late that even a small amount of infection on the lower parts of stems will prevent many of the pods from filling out.

OTHER ORGANISMS REPORTED AS PRODUCING ROOT ROT OR WILT

Fusarium vasinfectum Atk. was isolated only once from a diseased root collected near Blakely. It was not considered as a cause of any of the disease this year, and no experiments were conducted with it.

Phoma sp. was reported by Wolf (10, p. 128), as causing a stem rot at Auburn, Alabama. This was not found during this investigation.

Phymatotrichum omnivorum (Shear) Dug. was not seen.

SUMMARY

- 1. Fusarium martii var phaseoli is the causal agent of an early, or seedling wilt. This depleted the stand 10 to 38 per cent in Clay and Early counties in 1931. The injury is very slight on mature plants.
- 2. Bacterium solanacearum is the cause of an early wilt followed by stem- and root-rot-producing yellows in mature plants. Such plants are badly attacked by Diplodia natalensis or Sclerotium rolfsii. Bacterial lesions, with the plugging of xylem vessels in those regions, on lower stems and peduncles, prevented many of the larger types from filling out the pods.
- 4. Sclerotium rolfsii produces a serious root and peanut rot. It was most serious in fields that had much early wilt. This fungus, in 1931, developed most abundantly in August on sandy lands that were so infested last year.
- 5. Control appears to lie in varieties resistant to *Bact. solanacearum* and *S. rolfsii*.
- 6. The Alabama Runner is most resistant of the varieties adapted to Georgia and the White Spanish is most susceptible.

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OBSERVATIONS ON THE PARASITIC ABILITY OF CERTAIN SPECIES OF PYTHIUM

F. K. SPARROW, JR.

In the course of a morphological study conducted several years ago on certain species of Pythium the writer became interested in determining whether or not these fungi, all found in nature as parasites of algae, could attack other algae as well or even infect such very dissimilar hosts as flowering plants.

A few experiments of a rather preliminary nature were performed, the hope being that these would be supplemented in the future by a more exhaustive study. While neither the materials dealt with nor the data obtained were extensive, yet it has seemed desirable at this time to give a brief account of the results, even though they may be somewhat fragmentary.

MATERIALS AND METHODS

The 3 species of Pythium, P. dictyosporum Racib., P. angustatum Sparrow and P. adhaerens Spar. (5, 6), were all obtained from infected green algae, collected in the vicinity of Cambridge, Massachusetts, in 1926–27. Pythium adhaerens was found parasitic in Rhizoclonium hieroglyphicum Kütz., whereas the other 2 were isolated from Spirogyra crassa Kütz. Details of the method of isolation are given in the 2 previously cited papers by the writer.

The methods of fruit inoculation were similar to those outlined by Drechsler (2) and Harter and Whitney (4). Inoculation experiments designed to test the capacity of the fungi to produce root rot followed the method proposed by Miss Buisman (1), while Drechsler's technique was used in the damping-off work (3). In the latter instance eight 7-in. pots of sterilized soil were used for each of the plants (sugar beets and radishes). In each series 4 pots were inoculated with the fungus growing in Petri dishes of corn-meal agar and 4 were inoculated with corn-meal agar alone (controls). One hundred seed balls of sugar beets were planted in each of the pots, while, in the case of the radishes, a medium density of seeds was sown. By frequent watering a high degree of moisture content in the soil was maintained.

The technique employed in the inoculation of the algae was as follows: Twenty hanging-drop cultures (van Tieghem cells) were prepared, each of which contained a number of individuals of the alga in a large drop of sterilized water taken from the pond in which the organism was found. Before the cover glasses were sealed on, the algae in each of these cultures were carefully examined for filamentous fungi and the possible presence

of free zoospores. If such were found, the van Tieghem cells containing them were discarded. Ten cultures were inoculated by placing in the drop a bit of washed mycelium of the particular fungus taken from a pure culture grown in a liquid nutrient medium. The other 10 cells were not inoculated. After sealing the cover glasses, the cultures, both inoculated ones and controls, were either immersed in the same region of the pool from which the alga had been collected, or, if this procedure were not practical, were left under conditions of light and temperature that closely simulated the natural habitat.

In some instances balanced cultures of the alga contained in small jars were similarly inoculated and suitable controls maintained. However, the difficulties encountered in following the course of the infection and especially the detection of the infection, as well as the short time during which data were taken, made this method impractical. It was interesting to note, however, that the results were in entire accord with those obtained by using the hanging-drop cultures.

While the use of hanging-drop cultures would seem at first like placing the algae under very restricted and unnatural conditions, the time during which observations were made was so short, usually from 6 to 10 hours, that it is highly improbable that any marked lowering of the resistance of the organisms occurred. In fact, not only did the algae fail to show signs of lowered vitality but, on the contrary, cells in an active state of division were nearly always observed, even after several days in these cultures.

RESULTS AND DISCUSSION

The results of these experiments to determine the pathogenicity of the 3 fungi to various algae are presented in the following table.

The foregoing table discloses several interesting facts. None of the fungi, under the conditions of the experiments, was found to possess the ability to infect any large percentage of the various algae used. Pythium adhaerens was the most virulent of the 3, infecting a member of the Myxophyceae, 3 members of the Chlorophyceae, and one of the Chrysophyceae. It is interesting to note that the gelatinous sheath, often exceedingly thick, as in Microcoleus, Nostoc, and Batrachospermum, offered no resistance to the incursions of the 3 fungi, although the latter were unable to penetrate the cell wall. The ease with which P. adhaerens and P. dictyosporum infected Tolypothrix was rather unusual, in view of the fact that no members of the Oomycetes, with the exception of Resticularia, have been reported on any of the Myxophyceae.

In the following table are summarized the results of the inoculation of various fruits as well as the results of the root-rot experiments.

TABLE 1.—Results of inoculation of various algae with Pythium adhaerens, P. dictysporum, and P. angustatuma

	Inoculum						
Host	P. adhaerens		P. dictyosporum		P. angustatum		
	Inocu- lated	Con- trol	Inocu- lated	Con- trol	Inocu- lated	Con- trol	
Nostoc sp. (%)	-	-	<u>-</u>	_	-	-	
Oscillatoria sp. (?)	-	-7-17		_	-	-	
Tolypothrix sp. (?)	+	-	+	-	_	_	
Microcoleus sp. (?)	_	- -		_	_	-	
Rhizoclonium hieroglyphicum	+b		+	_	+	n	
Spirogyra crassa	+	_	+p		+6		
Ulothrix zonata Kütz	+		_	-	-	-	
Vaucheria sessilis DC.	-	,		-	_	_	
Oedogonium sp. (?)	_	<u>-</u>	-1-	-	+		
Closterium acerosum	_	_	-		-	- 1	
Nitella flexilis	_		_	-	_	-	
Synedra sp. (?)	+	-	-		_	-	
Batrachospermum moniliforme Roth	_		_	_	_	_	

^a A plus sign denotes that infection of algae occurred in all of the inoculated hanging drop cultures; a minus sign indicates that no infection occurred.

b The host on which the fungus was found in nature.

From an examination of table 2 it is evident that in the majority of cases the fungi are incapable of living parasitically on the fruits used, at least so under the conditions of the experiments. In 8 instances, however, complete destruction of the inoculated fruits resulted. Five of these cases, which included representatives of the Solanaceae and Cucurbitaceae, were producted by Pythium adhaerens, the species also found to be most successful in parasitizing various algae. The 3 varieties of cucumbers were the most susceptible of all the fruits used, being attacked by both P. adhaerens and P. dictyosporum. The rot produced by the fungi on the various fruits was of a "leak" type, a watery exudation accompanying the disintegration of the tissue.

With respect to the root-rot experiments, it may be noted that the varieties of the 2 plants used, under the conditions of the experiment, were susceptible to *Pythium adhaerens*. In addition, Mammoth White Cory

TABLE 2.—Results of fruit-rot and root-rot experiments

	Inoculum						
Host	P. adhaerens		P. dictyosporum		P. angustatum		
	Inocu- lated	In- fected	Inocu- lated	In- fected	Inocu- lated	In- fected	
Virulence to fruita							
Purple plums	6	0	6	0	6	0	
Snap beans	30	0	30	0	30	0	
Tomato (green and ripe)	14	14	12	0	12	0	
Eggplant	4 b	0	2	0	2	0	
Green pepper	4	0	4	0	4	0	
Cucumber Granite State White Spine White Wonder	$\begin{array}{c} 12 \\ 12 \\ 8 \end{array}$	12 12 8	$\begin{array}{c c} 12 \\ 12 \\ 6 \end{array}$	12 12 6	6 6 4	0 0 0	
White Summer Crookneck squash Yellow Summer Crookneck	4	0	4	0	2	0	
squash	4	4	4	0	2	0	
Des Moines squash	4	0	4	0	2	0	
Pattypan squash	4	0	4	0	2	0	
Ability to produce root rote							
Corn (Mammoth White Cory)	21	15	12	0	12	5	
Pea (Breck's Old Glory)	21	10	12	0	12	0	

a In all cases of fruit rot the same number of controls were used as the number

inoculated. No cases of infection by a Phycomycete were observed.

b All fruits finally became invaded by various fungi imperfecti. Careful examina-

tion showed no phycomycetous mycelium in the infected parts.

corn was parasitized by P. angustatum. Pythium dictyosporum, however, was unable to affect the roots of either peas or corn. The infected roots were easily recognized externally by their glassy brown aspect.

It may be justly argued that, inasmuch as pea and corn plants are not aquatic, they were cultivated under circumstances unfavorable for them but quite favorable for the fungi. This question has been considered by Miss Buisman (1) with whom the present writer is in agreement. She says, in part, "Phycomycetes . . . are water-fungi and easily produce swarmspores in mineral solutions, so that in water-cultures the circumstances are very favorable for infection. The pea seedlings, however, grow so well in v. d. Crone's culture solution, and produce such a healthy root system, that according to my opinion, they may well be compared with plants grown

Nine control plants were used in each instance, all of which were in a healthy. vigorous condition at the termination of the experiments.

under normal circumstances. At the utmost one may get an exaggerated idea of the pathogenicity of the parasite . . . ''.

The results of the attempts to produce damping-off in sugar beets and radishes by inoculation with the 3 species of Pythium are summarized in the following table.

TABLE 3.—Percentage of infection resulting from artificial inoculation of sugar beet and radish with Pythium adhaerens, P. dictyosporum, and P. angustatum².

	Inoculum						
${f Host}$	P. adhaerens		P. dictyosporum		P. angustatum		
	Inocu- lated	Con- trol	Inocu- lated	Con- trol	Inocu- lated	Con- trol	
Sugar beets ^b (Klein's Wanz- leben)	Per ct.	Per ct.	Per ct.	Per ct.	Per ct.	Per ct.	
Radish (Rice's Early Scarlet Globe)	0	0	0	0	0	0	

² Expressed in a percentage of Pythium-infected seedlings of the total number of seedlings. Those infected by *Phoma betae* were *not* counted.

It may be seen from the foregoing table that of the 3 fungi only Pythium adhaerens was capable of producing damping-off, and that, only of sugar beets. There was no question of the pathogenicity of the fungus to this plant for every one of the damped-off seedlings contained not only mycelium but numerous oospores of the parasite. The development of the asexual stage of this fungus was easily induced by immersing infected seedlings in water for a few days. In view of its virulence to this host, it seems probable that, upon further investigation, P. adhaerens will be found capable of producing dampingoff in other hosts.

In conclusion, the writer wishes to reiterate that these few experiments cannot be considered as final but are rather of a preliminary nature. For example, with respect to the infection experiments on algae, it is quite possible that varying environmental conditions may make the alga more susceptible to the fungus at one time than at another. It is hoped that an opportunity will be afforded in the future to make a more exhaustive and more precise study of the pathogenicity of these fungi.

The writer wishes to express his thanks to Dr. F. A. Guba of the Massachusetts Agricultural College Experiment Station, at Waverly, for

b In all cases a very small percentage of the plants were attacked by Phoma betae.

his many courtesies, to the Director of the Biological Laboratory, Long Island Biological Station, for the use of the facilities of that institution, and especially to Prof. W. H. Weston, Jr., under whose guidance this work was done.

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PHYTOPATHOLOGICAL NOTE

A correction.—In the paper entitled "Immunity in Plants" (Phytopathology 22: 95–102. 1932) the following statement on page 97: "In fact, Hursh, among others, in a study of a large number of wheat plants, observed that a parallelism exists between resistance and acidity as expressed in terms of pH (13)" should read instead: "... in a study of wheat plants, observed that no parallelism exists between ..."—J. A. DE Tomasi.



BOOK REVIEW

Hubert, Ernest E. An Outline of Forest Pathology. v-viii+543 pp., 168 figs. John Wiley and Sons, Inc., New York; Chapman and Hall, Limited, London, 1931. Price, \$6.00.

Professor Hubert's textbook of forest pathology is the first of its kind produced in America. Fortunately for the undertaking, the author is well equipped by breadth of experience in research and knowledge of forestry to perform this much-needed service. The work is predominantly American in the sense that it deals particularly with the materials and the pathological problems of the forests and the forest products of the United States and Canada. Many results of the observations and the investigations of the pioneers in American forest pathology, supplemented by applicable European data, are now made conveniently accessible and the sources of information are supplied by copious references to the literature.

The book is divided into 3 main sections, entitled: Part I. Introduction (pp. 1-106); Part II. Forest Pathology (pp. 107-445); Part III. Wood Pathology (pp. 446-531).

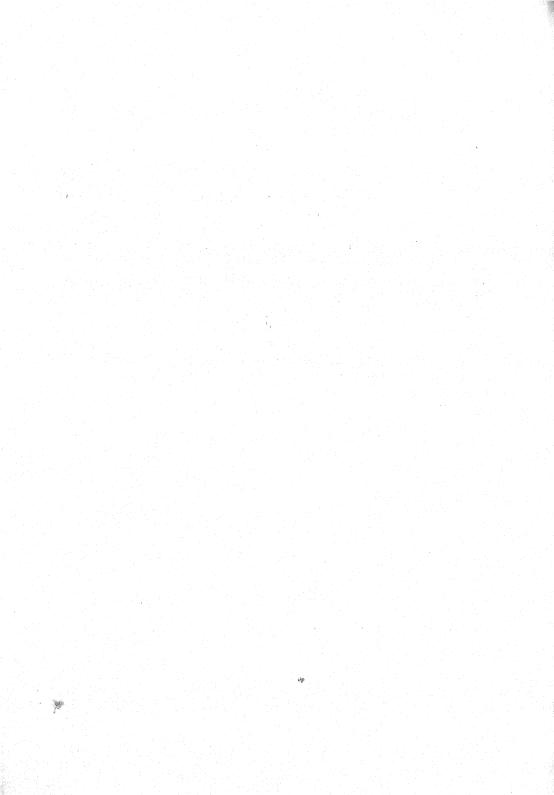
Part I consists of 7 chapters. Chapter I is headed "Historical"; aside from a brief reference to earlier studies on decay in wood, it consists mostly of a list of some of the forest pathologists, past and present, and a brief outline of the organization of forest-pathology services in the United States. Chapter II, The Trend in Forest Pathology, is more correctly a brief, incomplete, historical sketch than a clear exposition of the author's thesis. Chapter III, entitled the Classification of Tree Diseases, is prefaced by a definition of forest pathology "as the science of tree diseases, their prevention and control," a definition that is not satisfying. It is too broad in that it does not exclude orchard and ornamental trees and too casual in that it fails to mention the forest as the central interest, often the sole unit in prophylaxis. The author more accurately reveals his real conception of the subject in Chapter V, The Relative Importance of "Tree" Diseases, where, in passing, he criticizes mere interest in individual trees rather than "carefully planned and premeditated study of a large group of trees or of a forest." The table presented at the close of the third chapter is mainly a classification of agencies of disease rather than of diseases themselves. Chapter V very properly emphasizes the desirability of appraising the relative importance of forest ("tree") diseases in harmony with the aims and practices of sound forestry. The citation of "the white pine blister rust and the chestnut blight" as "the most important of all of our tree diseases" may be misleading in a way not intended. The latter, because of the thorough destruction it wrought, is now relatively unimportant in forestry. As for the former, one may well hesitate to give it first place on the list if such wide-spread and often wholesale diseases are considered as those caused by such fungi as Trametes Pini and Fomes igniarius, involving as they do almost the entire range of our forests, softwood and hardwood. These and certain others render entire stands of vast area unsound, often to a degree of utter worthlessness. Chapter VI concisely sets forth the economic losses from forest diseases. The final chapter of Part I, Symptoms, devotes much space to an exposition of the etiology of decay in trees and in structural timber. The first part of this long chapter presents an analytical key to diseases of forest trees (11 pages) based on external symptoms. While duly recognizing the originality of this scheme, experience alone can demonstrate its usability. Hartig and others have accomplished essentially the same purpose by including at the end of their texts lists of diseases, with their obvious symptoms under alphabetically arranged host names. Such lists have the advantage of brevity and directness.

Part II is devoted to a detailed description of forest diseases; the subject matter is comprised in 3 chapters, Chapter VIII dealing with those caused by "non-organic or physiogenic agencies," Chapter IX with those caused by "organic agencies," and Chapter X with "control." Chapter IX, (pp. 142-439), by far the longest in the book, is arranged according to "the taxonomic order of the causal organism." As might be expected, the treatment of the many known diseases is uneven, partly by design and partly through necessity. There are great differences in the relative importance of forest diseases, and the vast majority have so far scarcely been studied at all. Turning over the pages, one is struck by the large numbers that have been seen, and even more so by the fewness of those that have been investigated. In dealing with subject-matter of this kind an author is confronted with the choice of 2 methods, namely, commenting briefly on all, as Hubert, for the main part, has done, or limiting descriptions to a selected, representative few, treating each at considerable length and relegating the rest to bare enumerations at the ends of appropriate sections, as Professor Heald has done in his Manual of Plant Pathology. Possibly Professor Hubert's choice is wise, considering the matter at his disposal. Under the various topics discussed the author describes the symptoms, the etiology, and, wherever possible, known or likely methods of control. The text is abundantly interspersed with literature lists. It may be suggested that Chapters VIII and IX are not quite accurately titled, a suggestion that applies to several other chapters in the book.

Part III presents the subject of "Wood Pathology." Here the author is at his best. Part III falls into 5 chapters, namely, The Properties of Wood Affected by Decay, The Relative Resistance of Wood to Decay, deterioration caused by "saprophytic agencies" (dry rot, decay of timbers, etc.), deterioration caused by "semiparasitic agencies" (sap stains of

wood), and "control." Eventually, this subject will almost certainly demand a separate volume to itself. Just now, however, it is a welcome inclusion here, because there is yet no consistent treatment of it elsewhere.

All in all, An Outline of Forest Pathology is a creditable volume. It will no doubt be extensively used by students of forestry and will find a place on the reference bookshelf of others who are interested in conservation, tree surgery, wood preservation, pulp and paper manufacturing, etc., as hoped by the author. As a contribution to forest pathology, proper, in America, it shows that enough already has been accomplished to place the subject on an established footing. We now know that a multitude of diseases of one kind or another threaten all forest age classes: even certain butt or heart rots are known to infest comparatively young stands; the maiority of these diseases await investigation, especially from the standpoint of control. It is reasonable to anticipate that concentrated research on them, one after the other, will reveal both their etiology and practical methods of control. Obviously, a wider acquaintance with diseases of forest trees, forests as units, and forest soils is bound to be essential to the highest type of forestry practice as applied to reforesting, conservation, and utilization.—J. H. FAULL, Arnold Arboretum, Harvard University, Boston, Mass.



PHYTOPATHOLOGY

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SOME PATHOLOGICAL STUDIES ON APPLE CANKERS

NELLIE A. BROWN 1

INTRODUCTION

The perennial canker of apple trees was a well-known disease when the writer began to study a phase of it. It has the appearance of a very infectious type of disease, for the cankers spread deep in and along the stems and may be so numerous and large that a third of the tree is involved (Fig. 1, A and B). It is known only in the Pacific Northwest and such varieties as Yellow Newtown, Ortley, Jonathan, Esopus Spitzenburg, and Winter Banana are susceptible to it; the Northern Spy, Winesap, and Delicious are, in general, not considered susceptible. In spite of the fact that fruit from diseased trees has been shipped elsewhere, the disease has not been spread to the East. The cultivated apple and very rarely the pear are the only hosts known.

In 1925 Zeller and Childs (11) showed that what was thought to be anthracnose, produced by the fungus Neofabreae malicorticis Cord., was really 2 canker diseases instead of 1. They gave the name perennial canker to the second type, pointed out the differences between the 2 cankers, and described an organism producing perennial canker and named it Gloeosporium perennans. It is not within the scope of this paper to deal with what is considered the anthracnose type. It is sufficient to state that in these investigations if the sori on a canker had distinctly hooked spores, the distinguishing feature of anthracnose, instead of the curved spores of perennial canker, the data obtained in connection with it are not included in this paper. If there were no fruiting bodies on the canker, rarely any difference could be detected by the writer between anthracnose and perennial canker and, so, the canker was classed as the latter. When perennial canker was first described it was thought the organism remained in the tissue and continued to infect it, producing the new canker growth from year to year. Now it is thought by some workers that the callus tissue around the old canker has to be reinfected every year, therefore it is really an annual disease instead of a perennial, and to produce the canker advance or new cankers elsewhere on the tree there must be some other agent or condition to help bring it about.

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Fig. 1. Young and old stages of apple canker, Hood River Valley, Ore. $\times 3$. A. Canker on stem of Ortley apple. Three sets of isolation plates were made from this canker in December, 1928. No Gloeosporium colonies appeared in 2 of the sets; but 4 Gloeosporium perennans, 2 Penicillium, 24 white bacterial (all same species), and 19 yellow bacterial colonies (all same species), appeared in the third set. B. Advanced stage of canker on Yellow Newtown stem of apple.

In the winter of 1928–29 when the present work was undertaken, plant pathologists believed perennial canker to be a true parasitic disease because it has all the appearances of a disease induced by an active parasite. Entomologists who studied the cankers believed that the woolly apple aphis was the primary cause of the disease and that the fungus usually followed severe aphis infestation. Both groups of workers had evidence to back up their claims and consequently there was confusion as to methods for prevention or control of the disease. Papers dealing with the evidence to support their views were published by Childs (1, 2), Cooley (3, 4), Fisher (7, 8), Miller (3), McLarty (10), Reeves (7, 8), and Shear (4).

In 1930 Cooley and Miller (3) reported "that the margins of healthy calluses may be injured by cold and that canker infection readily takes place in the winter-injured margins of pruning wound calusses." Winter injury as the primary cause of perennial canker was brought out further by Cooley and Shear in 1931 (4).

The same year (1930) entomologists and plant pathologists of British Columbia concluded that perennial canker is found principally in districts where the woolly aphis is prevalent and in varieties subject to winter killing. Güssow (9) states: "Cankers may be more or less numerous depending upon the resistance of varieties to winter injury and when and where unusually prevalent this condition can almost without exception be traced to susceptibility to winter injury as well as to the presence of woolly aphis." He considers the aphis to be the carrier of Gloeosporium perennans.

PURPOSE OF THIS INVESTIGATION

The work reported in this paper was undertaken with a view to making a study of the location and extent of the fungus *Gloeosporium perennans* in the tissue of the apple cankers and the rapidity with which it spread in apple stems. Material was sent to Washington, D. C., from the Hood River Valley, Oregon, and the first part of the work was done in Washington. Isolations were made from many cankers of various sizes and ages and from different parts of the cankers in order to recover *G. perennans* from various parts of cankered areas. Part of this material was prepared for sectioning to study the presence and extent of the fungus in stained tissues.

VARIOUS ORGANISMS FOUND IN CANKERS

Repeated isolations from the inner bark and wood of cankers brought out the significant fact that had not been suspected—that Gloeosporium perennans was not always present in the canker tissues and that some other fungus, such as Alternaria, Fusarium, Cladosporium, or Dematium, was frequently present instead. The Dematium had a bacteria-like-colony growth and seldom developed any mycelium in plate cultures. Often pure cultures of a white bacterium occurred on the isolation plates, and sometimes there would be a mixture of more than one fungus or of a fungus and the white bacterium. The media used for the isolation plates included potato-dextrose agar. Corn meal, malt, Dox, apple, and beef agars also were used. Potato-dextrose agar, however, was found to be the most satisfactory. Using fall and winter material, the white-bacterium and Dematium colonies were the most abundant on the isolation plates whether alone or with some fungus. Fungi other than Gloeosporium occurred in the plates so frequently that they could not be considered chance invaders. The white bacterium frequently threw a yellow sport. The isolations were made before the sections were stained, and were studied to be certain that the cankers under investigation contained the fungus G. perennans and that all data relating to the position and extent of this fungus in the tissues, when studied in sections, could be referred to as G. perennans.

The continued appearance of bacteria and the various fungi on isolation plates and the frequent absence of *Gloeosporium perennans* made necessary a change in the work that had been planned. While it was still of value to study the location and extent of an invading organism in the diseased apple stems, the presence of so many different organisms made it necessary to make many isolations from cankers collected in various localities. This procedure prevented drawing a conclusion from results obtained in any one locality. Up to this time there had been no definite question among plant pathologists interested in the problem regarding the pathogenicity of *G. perennans* as the cause of perennial canker of apple trees.

INOCULATIONS WITH GLOEOSPORIUM PERENNANS IN ORDER TO PRODUCE YOUNG CANKER TISSUE FOR STUDY

In order to study the apple-stem tissues during the first stages of canker infection, inoculations were made into young apple trees. Seedlings 1 to 2 years old from Jonathan seeds, growing in pots in the greenhouse in Washington, D. C., were inoculated with pure cultures of *Gloeosporium perennans* by means of needle pricks in February and March, 1929. The trees were kept at various temperatures on the benches and in moist chambers, but no disease could be induced. Inoculations were also made with spores taken from mature sori on cankers. Thirty-three seedlings were inoculated with pure cultures and 13 with spores taken from sori on cankers. The seedlings were under observation for over 2 months. These results were negative also.

If Gloeosporium perennans were a true parasite and active in producing perennial canker in the Pacific Northwest, it would seem that it could scarcely fail to produce some infection in the East under these various conditions. The fact that it did not produce infection together with its infrequent occurrence on plates poured from the interior of cankers, led the writer to think that something else must feature in the production of the disease.

ISOLATIONS IN WASHINGTON, D. C., FROM CANKERS SENT FROM HOOD RIVER, OREGON

In December, 1928, and in January and February, 1929, platings were made from old apple-canker tissue carrying into the plates pieces of the material that had been first washed in water, dipped in alcohol, then in mercuric chloride 1–1,000 from 15 to 60 seconds, washed again in sterile

water, and cut up in a tube of sterile water. Sori containing spores of Gloeosporium perennans were usually noted on the surface of the cankers.

From 17 sets of isolations comprising 136 plates no uniformity of isolation of *Gloeosporium perennans* resulted. Potato-dextrose and Dox agar and occasionally corn-meal agar or beef agar were used as media for the isolations. In 9 of the 17 sets, no *G. perennans* appeared on the plates. Various organisms, both fungi and bacteria, appeared, of which the white bacterium and Alternaria were the most abundant. The other fungi were Dematium and Fusarium.

Isolations from the button type of canker. There is a type of canker prevalent in Washington State designated as the button stage. As the name indicates, the lesion is button-shape; it usually varies in size from 5 mm. to 2 mm. across. It was thought perhaps this was the beginning stage of the disease and, if so, Gloeosporium perennans should be very abundant in it, and the other organisms found in old cankers should not be present.

Seven sets of isolations, including 83 plates, were poured from cankers of this type received from Wenatchee in March, 1929 (Fig. 2, B). There was no spring advance indicated in these lesions, and, as the tissue was somewhat dark, the lesions must have developed some months previous, possibly in the fall. The plates that were made with Dox agar, potato-dextrose, and beef agar were watched carefully and studied daily for 2 weeks.

The most abundant organism present was Dematium, numbering 224 colonies; the next most abundant was the white bacterium, of which there were 136 colonies. There were 38 colonies of *Gloeosporium perennans*; other types of bacterial colonies, 15; and other types of fungi, 11 colonies.

Several different organisms were usually isolated from each lesion; no canker produced a pure culture of any one organism.

These results did not substantiate the earlier idea of the writer that the button lesion was the initial stage of the disease. It seemed rather that the button stage was a case of arrested canker development in which the tree was able to overcome the disease because of favorable conditions. In these lesions, as in the large typical cankers, there was no certainty that the fungus *Gloeosporium perennans* was present in each of them, or, if present, that it was abundant. So, it seemed possible that there might be some other cause or special condition back of the presence of *G. perennans* or possibly some other organism was responsible for the presence of the cankers.

STUDY OF CANKER ADVANCE IN WASHINGTON, D. C.

As the writer's work on this problem was begun in Washington, D. C., in the late fall of 1928, and as the younger parts of the cankers had been developing since spring, the isolations in fall and winter had been made from rather old tissues. In view of the results the writer thought it ad-

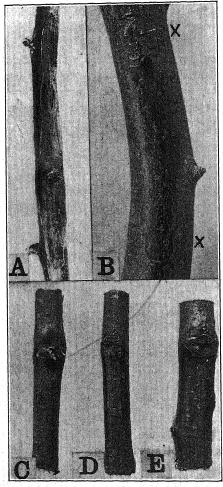


Fig. 2. Artificial and natural cankers on apple stems. A. Stem of Yellow Newtown apple inoculated with Newtown No. 1 (a white bacterium) June 3, 1929, and photographed Aug. 12, 1929. $\times \frac{1}{2}$. B. Button stage of canker from Wenatchee, Wash., shown at X. $\times 1$. Isolations made from these March, 1929. C-E. Stems of Yellow Newtown apple inoculated June 25, 1929, and photographed Aug. 10, 1929. $\times \frac{1}{2}$. C. Inoculated with pure culture of Gloeosporium perennans; D. Control punctures; E. Inoculated with pure culture of white bacterium (strain Newtown No. 1).

visable for further work to use the young canker tissue that starts in the spring to develop beyond the old canker. Thus, the dissatisfaction felt about isolating *Gloeosporium perennans* only infrequently from cankers could be cleared away with the use of young canker tissue, where its presence could be assumed to be certain. If one particular organism were the

cause of the disease, and that one G. perennans, it ought to be abundant in the advancing canker tissue.

Therefore, in the spring of 1929, an intensive study of young-canker advance was made with fresh material sent to Washington, D. C., from the Hood River Valley in Oregon. Freehand sections of this canker material were studied under the microscope; some of it was stained and studied, but no Gloeosporium perennans or other fungus organism could be located. Many isolation plates were made, but no fungus appeared on them. Frequently, colonies of the white bacterium previously noted appeared, but they were not numerous, as with isolations from old cankers. The work continued in Washington through April and most of May, and from 10 different platings, including 80 plates, no G. perennans or other fungi were isolated.

The canker advance in Oregon. Because of the failure to isolate Gloeosporium perennans from the canker advance in Washington, D. C., and the failure to induce infection on apple trees by inoculating with either pure cultures of that fungus or spores of the fungus taken from sori on cankers, the idea began to obtain that there must be some regional conditions in the West that bore an active part in the production of the disease. Consequently, further work with the canker advance was transferred to Oregon and continued there from the latter part of May to the middle of August, 1929.

In Oregon suitable canker-advance material for isolations was directly procured from the trees and used immediately, but with the same results as were obtained in Washington, D. C. Even when the youngest and freshest material available was used, no Gloeosporium perennans was isolated from the new advance of the cankers until the tissues had darkened and were dying. Then, as in the Washington, D. C., isolations from old cankers, other fungi besides G. perennans were found present in this tissue. Dematium, Alternaria, and Fusarium, and, in addition, Cladosporium and occasionally a Penicillium, were present. Twelve sets of plates were poured. The first fungus to appear on the plates poured from the canker-advance material was Alternaria, which appeared on plates poured July 16, 1929. G. perennans, Fusarium, and Dematium appeared on plates poured July 23, 1929.

The frequent appearance of the white bacterium on the isolation plates of both spring advance growth and old cankers made the writer suspect it might play a part in the production of cankers. So, this organism and the fungi associated with it so frequently on the plates poured from old cankers were used in Oregon for inoculations into susceptible trees. It might be stated here that in all the inoculations and injections made in Oregon the

stems were first sprayed with HgCl2 and then with water, or the stems were washed with HgCl, followed by a washing with water. The HgCl, was allowed to remain on for a minute or more to kill or weaken the various organisms that might be adhering to the surface before the inoculum was applied and before the punctures were made. Inoculations were made mostly with a fine needle. Each of the organisms, Fusarium, Alternaria, Dematium, and the white bacterium, together with its yellow sport, was able to produce canker lesions. The cankers produced by the white bacterium were the largest. When this organism was inoculated into an apple stem 1 to 5 years old, immediately after it was isolated from a canker, it produced a In 2 months a few of the fresh canker nearly 3 cm. long, in 3 weeks. lesions were as large as the natural cankers (Fig. 2, A). One produced in this way and sent to Washington, D. C., from which a water-color drawing was made, was 9 cm. in length. These cankers were produced on a Yellow Newtown tree, located in the last row in the orchard at the top of a hill and therefore exposed to extremes of temperature. The stems may have been weakened by winter injury, although it was not apparent on the surface. The white bacterium was reisolated from a portion of this canker in October, 1929, and again in May, 1931. The stem had been kept in the refrigerator at a temperature around 12° C. during the interval of nearly 2 years. No fungus colonies appeared on the reisolation plates. The culture of the white bacterium producing this canker was kept alive in subculture and its virulence tried out in the same orchard after it was a year old (1930). Lesions 3 cm. long were produced in 3 months. This was appreciably smaller than those produced the year before when the bacterium was first isolated.

For controls, 80 groups of punctures were made with a sterile needle on Newtown apple stems after first spraying them for 1 to 2 minutes with HgCl₂ 1–1,000, followed by a thorough spraying with sterile water. Seventy-five other groups of control punctures were made in which the surface of the stems was not sterilized. No lesions followed puncturing the sterilized stems, but 19 small cankers 6 mm. to 2 cm. long appeared on the non-sterilized stems in 2 weeks to 2 months.

The other apple stems inoculated in 1929 with the bacterial organism that produced cankers such as are shown in figure 2 were examined in 1930. No apparent increase in size was made during the spring of 1930. A fissure and callus surrounded the cankers on large stems; some of the small stems inoculated were dead probably because of winter killing through the canker openings. Some of the cankers on 3- to 5-year stems had healed except for central, small, dark areas, while a few on stems of the same age showed entire recovery. The inoculations with the other organisms had similar histories.

INOCULATIONS MADE INTO TREES IN A NONIRRIGATED ORCHARD

About 8 miles from Hood River there was an abandoned apple orchard on a hillside at an elevation of about 1,200 ft. that had never been under irrigation. The trees were 10 to 15 ft. high, and, although in poor condition, were bearing fruit. Three different bacterial species isolated from different cankers were inoculated into Jonathan and Esopus Spitzenburg trees the first of July to see if any infection might occur under the prevalent hot, dry conditions. No inoculations were made with fungi. Two strains of the white bacterium were used, 1 that had been isolated for over 6 months and the other less than 2 weeks. Eighty inoculations were made with the old strain, and 18 with the new one. No infections resulted from the former, but the latter produced 15 lesions, the largest of which was a little over 1 cm. long at the end of a month. The following spring the lesions were 2 to 4 cm. across and appeared as typical cankers. The development had occurred during the fall. Twenty-five inoculations made with the 2 other bacterial species were negative, as were the 67 groups of control punctures.

INOCULATIONS WITH A MIXED CULTURE

Shortly after it was first isolated, the white bacterium was inoculated into a Bartlett pear tree and small lesions developed on young twigs in 2 weeks. They did not enlarge, however, like those on the apple trees. The white bacterium in its active stage was mixed with a fresh isolation of Gloeosporium perennans and both pear and apple trees were inoculated with the mixed culture. Controls were held on both trees by inoculating with G. perennans alone and the white bacteria alone. This was done in July, 1929, and by the middle of August the largest lesion from the mixed culture on the apple tree was 2 cm. long. The largest on the pear tree was 1 cm. long. No observations were made again until the following April. At that time the cankers had developed further and were larger than those produced either by G. perennans alone or the bacterium alone. The largest canker from mixed inoculation on an apple tree was 3 cm. x 2½ cm. No advance had taken place in the spring and a callus had formed around the margins of the canker. The development must have been during the fall or winter. The controls on the apple tree made by inoculating with G. perennans alone produced cankers 1 to 11/4 cm. long; the control inoculations made with the white bacterium alone produced cankers 1 to 2 cm. long; both series produced smaller cankers than the inoculations with mixed cultures. The pear cankers produced by the mixed culture had also increased in size and were strikingly different from those inoculated with G. perennans alone or the white bacterium alone. There was no spring advance, however, and the development on the pear stem, as in the apple, must have occurred during the fall or winter. The largest individual canker was 2 cm. long. In the case of both the apple and pear trees several inoculations had coalesced, giving an appearance of a solid canker 4 to 5 cm. long.

The control inoculations on the pear tree, using the 2 organisms alone, bore the same relation to the results with the mixed culture as in the case of the control apple tree; that is, the mixed culture produced the largest cankers.

At the time the various other fungi were inoculated into apple trees, inoculations were likewise made with both old and new isolation colonies of Gloeosporium perennans. The colony that had been isolated the previous fall seemed inactive, but the one recently isolated produced cankers in 15 days (Fig. 2, C), although there was only a small percentage of takes. This was usually the case with all organisms tested; there was always less than 50 per cent infection (Table 1). Control punctures and inoculations with the white bacterium were made at the same time as the inoculations with G. perennans (Fig. 2, D and E). The fact that several organisms could cause similar lesions on the apple tree and that there were unknown factors that influenced the susceptibility of the stem continued to point to some physiological condition as an important factor in the causation of the disease. This conclusion was reached before the close of the summer of 1929.

INOCULATIONS IN OREGON IN 1930

The inoculations with the various organisms in Oregon, in 1930, were made almost entirely with the isolations of the year before. The organisms had been kept alive by subcultures during the interval, for, at the time, it was not thought necessary to make new isolations. Fresh isolations of these mildly parasitic organisms must, however, carry with them more pathogenic power, for the cankers produced by inoculations in the spring of 1930 were smaller than those produced in 1929 (Table 1). When examined in the summer of 1931² most of the cankers, which were recorded by number, had healed over and only a few millimeters of dead areas remained. Gloeosporium perennans was among those healed over. The 21 definite canker lesions that were present were mostly under $1\frac{1}{2}$ cm. long; the largest was 3 cm. Since the inoculations bearing these numbers were not recorded with lesions in the summer of 1930, they must have been slower developments that took place in the following fall. The largest canker lesions recorded in 1931 were from inoculations with the fungus Dematium.

² Detailed, careful notes were made for the writer in August, 1931, by Mr. E. V. Shear, Associate Pathologist, U. S. Fruit Disease Field Station, Hood River, Oregon.

1932]

TABLE 1.—Inoculations into Yellow Newtown apple trees in Oregon, in 1929a

Organism	Number of inoculations	Cankers produced	Size
Bacteria (4 species)	477	158	1 cm. to 15 cm.
Gloeosporium perennans	84	23	1 " to 2½ "
Fungi (2 other genera)	47	12	8 mm. to 13 "
Total	608	193	
Controls			
Stems washed with HgCl ₂ 1-			
1,000 and water	80	0	
Stems not washed with HgCl2			
1-1,000 and water	75	19	6 mm. to 2 cm.
Inoculations in Oregon in	1930		
Bacteria (4 species)	68	19	8 mm. to 3 cm.
Gloeosporium perennans	38	7	8 " to 2 "
Fungi, 7 others	231	64	6 " to 2½ "
Total	337	90	
Controls			
Stems washed with HgCl ₂ 1-			
1000 and then with water	33	0	
Stems not washed with HgCl ₂		no cankers but	
1-1,000 and then with water	25	some dead areas	
		4 to 5 mm.	

^a In 1929 80 per cent of the inoculated stems were wrapped with oiled paper in which a piece of moist cotton was enclosed. This was done because of the dry winds, which not only desiccated the inoculum but carried spore-laden dust.

In 1930 about 50 per cent of the inoculated stems (and injected stems in table 2) were wrapped with oiled paper and the ends of the wraps waxed to prevent evaporation and the entrance of aphids.

It may be stated here that almost no natural cankers occurred in the Hood River Valley orchards during the spring and summer of 1931, nor was there canker development from old cankers already present. The winter of 1930–31 was a mild one and the trees were not injured by cold.

ISOLATIONS FROM WINTER-INJURED CALLUS TISSUE

Apple-canker material was collected in January and February, 1930, in the White Salmon Valley, Washington, a few miles from the Hood River Valley in Oregon, and sent to Washington, D. C., for investigation. The calluses had been well formed but winter injury had killed the tissue at the edges of the callus. There were no sori present on the dead areas and

the specimens did not look like perennial canker. Some of the inner cambium was alive and some dead. Six sets of plates were poured from the winter-injured callus of several stems, and from these plates Fusarium, Gloeosporium, Alternaria, and the white bacterium were isolated. Alternaria was the most abundant. Winter-injured callus on apple trees, it seems, may have one or more organisms present in the dead or dying tissue, among which Gloeosporium perennans may or may not be present.

Isolations from cankers on small apple stems. Stems ½ inch in diameter from Hood River Valley, with canker wounds extending halfway through them, were received in Washington, D. C., in January, 1930. Plates were made, using the inner bark and the wood of the cankered tissue. Pieces of material were sterilized ½ to ¾ min. in HgCl₂, washed in sterile water, and placed on hardened potato-dextrose agar. Fifty small pieces of the wood from the cankers were treated in this way and 40 pieces of the inner bark. The plates were observed for 12 days. Gloeosporium perennans grew from 4 of the 50 pieces of the wood tissue, Alternaria from 2 pieces, and the white bacterium from 4 of the 50 pieces of wood. The rest of the pieces were sterile. The organisms growing from the inner bark were as follows: G. perennans grew from 5 of the 40 pieces, Alternaria grew from 3, and the white bacterium grew from 12; the remainder of the pieces were sterile.

EXPERIMENTS WITH CANKER FILTRATES, ENZYMES, ETC.

Because the definite canker-advance tissues did not contain the fungus Gloeosporium perannans or other fungus until July or when the cankeradvance tissue had darkened and appeared to be dead or dying, it was thought that perhaps this advance might be due to the action of an enzyme produced by the fungus present in the old part of the canker, or else by a by-product of the dead canker tissue. To test this some typical cankers were ground up, mixed with water, and filtered through a Chamberland filter. The filtrate tested for sterility by the poured-plate method was found to be sterile. Some of it was then injected into stems of susceptible apple trees. Likewise, enzymotic material was isolated from crushed cankers, dissolved in water, and injected into stems of susceptible apple trees. In addition, liquid cultures of G. perennans separated from the fungus growth and filtered through a Chamberland filter were also injected into apple trees. The filtrate from carrots rotted with Bacillus carotovorus was also injected into apple trees, and sterile water into other apple trees for controls. No cankers occurred from a total of 350 injections that were made into trees, both in Virginia and Oregon (Table 2). With the carotovorus filtrate 6 of the 52 injections made in Oregon produced lesions with dead tissues ½ to 1 cm. in length and 4 to 5 mm. wide, in a little over a month. They were not cankers but showed that some of the apple-stem tissue was affected by the presence of this filtrate.

INOCULATIONS IN VIRGINIA IN 1930-31

Jonathan apple trees in Virginia were inoculated with the same organisms used in Oregon. The fungus Gloeosporium perennans was not used for inoculations as the belief was prevalent that it was the sole cause of perennial canker of apple trees and therefore should not be introduced into the Eastern States. However, 2 other Gloeosporiums were used—G. cyclaminis and G. musarum. The inoculations were made each month of the fall and winter of 1930 and the spring of 1931, and continued through the month of May. The work was repeated monthly in order to subject freshly inoculated stems to various weather conditions to see what the effect might be. A total of 1,401 inoculations were made with 219 groups of control pricks made with a sterile needle. Some of the inoculated and control stems were wrapped with oiled paper, others left unwrapped.

No canker was produced by any of these inoculations in Virginia (Table 3). Except in a relatively few cases, there was no evidence on the surface of the stems of any reaction from the inoculations. However, when the bark was cut into at the inoculation places there was usually a darkened area that did not extend into the wood, or, if it did, only to a very slight extent. The bark evidently acted as a medium for the organism to live in for in every case where reisolations were made from this dark tissue the organism could be recovered. Reisolations were made and the organism recovered in the case of 9 different organisms. There were 19 cases where there were indications of tiny lesions on the surface. The tissue had sunk a little and was darker than the surrounding tissue for a centimeter or less, and in a few cases this area was outlined by a fissure. These few simulated what was designated in Washington State as the button type of canker. Seventeen of the 19 lesions came from inoculations made in October and November; the other 2 were from March inoculations. Both fungi and bacteria produced these little lesions. Darkened areas usually surrounded the control punctures but they were only a few millimeters in extent. The fungi and bacteria evidently used the bark of the Jonathan trees in Virginia as a medium on which to live but did not produce cankers. tions were made from the discolored inoculated areas 1 to several months after inoculations were made, and, as stated above, the organism could always be recovered.

DISCUSSION

The frequent absence of Gloeosporium perennans from a typical perennial apple canker as well as the frequent presence of various other organ-

TABLE 2.—Hypodermic injections into Yellow Newtown apple trees in Oregon in 1930, with various sterile solutions, etc.

Material used for injections	Number of injections	Number of lesions	Size and character of lesions
Fittrate of crushed canker In Oregon '' Virginia Total	56 22 78	0 0 2	A few mm. long and no appearance of canker
Filtrate of liquid culture of Gloeosporium perennas In Oregon 't' Virginia Total	$\frac{75}{25}$	5 0 0	A narrow line of dead tissue form. to 1 cm. long unlike canker in appearance
Filtrate of Bacillus carotovorus culture In Oregon '' Virginia Total	52 12 64	9	{5 mm. to 1 cm. long and 4 to 5 mm. wide
Enzyme from apple cankers dissolved in water In Oregon '' Virginia Total	$\frac{82}{25}$	$\frac{37}{0}$	A narrow line of dead tissue 5 mm. to 2½ cm. long
Sterile sand in water In Oregon '' Virginia Total	43 none 43	30	A narrow line of dead tissue 4 mm, to 1 cm, long
Controls Sterile water In Oregon '' Virginia Total	$\frac{25}{10}$	000	
Liquid synthetic medium	23	Small splits with a	

TABLE 3.—Inoculations into Jonathan apple trees in Virginia in 1930-31a

Organism	Number of inoculations	Number of cankers	Surface markings
Bacteria (4 species)	406	0	7 with dark areas 1 cm. and less
Fungi (6 genera including 2 Gloeosporium species but not G. perennans)	995	0	12 with dark areas 1 cm. and less
Controls	219	0	

^a About 50 per cent of the inoculated stems were wrapped with oiled paper; the ends of wraps were not waxed.

isms seemed to indicate that there is some other underlying factor, possibly a physiological one, that is responsible for the disease. The fact that various unrelated organisms could be repeatedly isolated from cankers and that a number of these could artificially reproduce cankers, indicated that the plant tissue must be weakened, a condition brought about by one cause or a combination of causes. The most outstanding and important one seemed to be the condition in which the trees were left after sudden low temperatures in the winter.

The hard freeze of the early part of the winter of 1919-20, when the temperature in the Hood River Valley and other Northwestern apple regions fell to -27° F., produced wide-spread damage in the apple orchards of those The dead and dying tissues that resulted from the freeze were a good medium for the growth and development of Gloeosporium perennans, other fungi, and bacteria. G. perennans, a weak parasite already common in the region, producing fruit rot in ripe apples (6), became rampant in these orchards. Due to the heavy and widespread destruction of trees or parts of trees by this freeze, the orchards did not get cleared of the dead branches for a year or 2 or even longer. Up to that time the canker was little known and was not feared. The dying branches proved a menace and gave the opportunity for a widespread development of the spores of G. perennans, and possibly successive contacts of successive generations of spores with dead apple bark increased its tendency to pathogenicity. The same can be said of the other fungi and bacteria taking up their abode in the dead apple tissue.

Since the winter of 1919-20 it has been noticed that perennial canker is more prevalent and serious in those regions where lower temperatures prevailed the previous winter. The climate of certain fruit-growing sections of the Pacific Northwest is generally mild throughout the winter, with the

exception of occasional hard freezes, which do not occur every year. The winters of 1924 and 1926 were also such severe ones that many trees were weakened and later became prey to this disease. Because of the generally mild climate, the trees do not get hardened off gradually to meet the cold, as in the apple-growing sections of the East. Thus unprepared, the trees, in a more or less succulent condition, become injured to varying degrees by the cold. If the injury is not sufficient to kill, there are devitalizing effects from the cold that make the tissues susceptible to disease. During the period of this investigation there was no typical canker year; if the inoculations with the various organisms had been made after a very severe winter it is quite probable that many more canker lesions would have been produced.

In the literature about perennial canker of apple trees previous to 1930 the possible effect of winter injury is mentioned frequently (1, 2), and in 1930 Fisher and Reeves (7) definitely advised orchardists to prepare their orchards to resist freezing weather because by so doing the trees would be better able to resist perennial-canker infection. They made recommendations for bringing the trees to a hardened condition.

A few of the orchardists in the Hood River Valley already claim to have cured badly cankered trees by proper feeding and their fear of future losses from cankered trees has vanished. If their claim still holds after a few more years have elapsed, the feeding, irrigation, and cultural methods of these orchardists should be put into general use.

According to entomologists, perennial canker usually follows a woolly-aphis infestation; the tissue of young apple stems and the callus of pruning wounds are weakened by the attack of the aphids, permitting the entrance and advance of the *Gloeosporium perennans*. It is doubtless true that woolly-aphis infestation on young callus tissue weakens it so that it is more readily injured by cold weather and later by cankers.

Sprays have been ineffective in controlling the disease, though they no doubt cut down the number of active organisms on the surface that could enter the tissues following winter injury. Crenshaw (5) has thrown light on the effectiveness of sprays by showing how long they can prevent germination of *Gloeosporium perennans* spores under ordinary orchard conditions.

SUMMARY

The fungus Gloeosporium perennans, heretofore considered the organism causing the perennial canker of apples in the Northwest, was found frequently absent in otherwise typical cankers. Other fungi were present, also bacteria, sometimes in company with G. perennans but just as frequently without it.

These organisms were isolated from typical apple cankers and were inoculated into apple trees in Virginia and in the Hood River Valley, Oregon. No cankers were produced in Virginia, but typical ones were produced in Oregon with Alternaria, Fusarium, Dematium, Cladosporium, Penicillium, and a white bacterium. *G. perennans* did not infect more readily than the other organisms, but field inoculations were made with it only in spring and summer. In some cases there was canker growth during the fall following inoculations made in the spring of 1929, but there was no new canker development the next spring. The inoculations made in the spring of 1930 from year-old isolations of bacteria and fungi were mostly healed over when examined in 1931. Those canker lesions present were small and apparently had developed in the fall from the spring (1930) inoculations.

G. perennans was not used for inoculations in Virginia because of the danger of introducing the disease in a region where it never had occurred. Other species of Gloeosporium, G. musarum and G. cyclaminis, however, gave negative results when used in Virginia.

In looking for a reason why cankers formed from inoculations made in Oregon and none from inoculations made in Virginia, the writer has concluded that the reason is a local one. There was evidently no active parasite connected with the disease, even though the appearance of the cankers belied it.

It has been noticed since the cold winter of 1919-20 that perennial canker was more extensive in those regions where lower temperatures prevailed. So, judging from field observations made and from evidence obtained from other workers, together with the fact that no cankers occurred following the mild winter of 1930-31, it is concluded that the disease is primarily due to winter injury with woolly aphis as a factor in the injury. The organisms that live in the cankers following this injury could not gain foothold and develop in healthy apple tissue, but the changed conditions in the cells of winter-injured tissues offer a good medium for their growth and development.

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ZINC-LIME: A FUNGICIDE FOR THE PEACH

JOHN W. ROBERTS AND LESLIE PIERCE1

INTRODUCTION

The first successful fungicide for general use on the peach during the growing season was the so-called self-boiled lime-sulphur mixture introduced by Scott (10) in 1907. Since that time many substitutes for this mixture, all containing elemental sulphur as the active ingredient, have been introduced and successfully used. These sulphur fungicides are excellent protectants against attacks by the brown-rot fungus, Sclerotinia fructicola (Wint.) Rehm,² and the scab fungus, Cladosporium carpophilum Thüm., but are not effective against the bacterial-spot pathogen, Bacterium pruni E. F. Smith.

In 1925, at the request of the Purdue University Agricultural Experiment Station and the Knox County Horticultural Society, the writers undertook to develop, at Vincennes, Indiana, a spray that would control peach bacterial spot. Since susceptibility to the disease extends over a long period, it was evident that many applications of spray would be necessary. It was also evident that a fungicide applied so often would have to be practically noninjurious to the peach to avoid a serious accumulation of spray injury. Of the 200 mixtures and combinations tested, one, composed essentially of zinc sulphate and hydrated lime in water and referred to in this paper as zinc-lime, has consistently given the best results. The writers first reported upon this mixture as a control for bacterial spot of peach in 1929 (2). More complete reports were later published in the Proceedings of Horticultural Societies (3, 4, 5, 6, 7, 8) and in the American Fruit Grower Magazine (9). These papers reported results with zinc-lime on the control of bacterial spot and stimulation of the peach. They also reported favorably on the use of arsenate of lead with zinc-lime but reported no decisive results on experiments for the control of scab and brown rot.

This paper is a report of experiments to determine the possible usefulness of the spray as a complete fungicide for use on peaches during the growing season. The term "fungicide" is here used to include substances toxic to the organisms causing bacterial spot, seab, and brown rot, and non-injurious or relatively noninjurious to the peach in active growing condition.

1 The writers thank members of the Knox County, Indiana, Horticultural Society and the Purdue University Agricultural Experiment Station, particularly M. W. Gardner, C. L. Burkholder, and Arthur Goss, for their cooperation and encouragement in the development of this spray.

² Also named S. americana (Wormald) Norton and Ezekiel and considered by some as not specifically distinct from S. cinerea (Bon.) Schröt.

THE ZINC-LIME SPRAY

The name zinc-lime is applied to a mixture of zinc sulphate, lime, and water. Zinc-lime, 4-4-50, denotes a mixture of 4 lbs. of zinc sulphate $(ZnSO_4 \cdot 7H_2O)$ and 4 lbs. of hydrated lime in 50 gals. of water. The name zinc-lime was adopted after several trials. First, the name zinc-Bordeaux-mixture was tried but was abandoned because it was taken by some to indicate the presence of a copper salt. A trial of the name zinc sulphate spray caused one grower to omit the lime, with, of course, disastrous results. The name zinc-lime was then adopted because it is short, easily remembered, and suggests the composition of the spray.

As first used by the writers, either casein-lime or alum was added to zinc-lime. Gradually, however, the simple formula 4-4-50, without an additional substance, was adopted. The casein-lime was dropped because it seemed not to lessen the settling time of the precipitate and not to add to the sticking and spreading qualities of the mixture. Alum, when added to the mixture, produces a precipitate that lessens the settling time, but in laboratory tests the addition of an alum greatly decreased the toxicity of zinc-lime to conidia of the peach brown-rot and scab fungi. In these tests zinc-lime, 4-4-50, prevented germination of brown-rot conidia and stopped the growth of germ tubes from scab conidia directly after germination. Zinc-lime, 4-4-50, to which alum was added, did not prevent the germination of brown-rot conidia, though it inhibited somewhat the growth of germ tubes and only slightly checked the growth of germ tubes from scab conidia.

The substitution of sodium carbonate for hydrated lime or of zinc chloride for zinc sulphate has resulted in severe injury to the peach.

The following is recommended as a standard formula for the zinc-lime spray, but the proportions of zinc sulphate and hydrated lime to water may be increased at least to 8-8-50 if desired, without causing injury. However, proportionately better results have not followed the use of a mixture stronger than the one here recommended.

Zinc sulphate	4 lbs.
Hydrated lime	4 lbs.
Water	50 gals.

Zinc sulphate (ZnSO₄·7H₂O), sometimes called granular zinc sulphate or white vitriol, is a common chemical easily obtained. A so-called anhydrous zinc sulphate is sometimes found on the market. This product is not anhydrous but contains, in varying quantities, less water than the granular zinc sulphate and is more easily handled because it is relatively free from lumps. For practical purposes, 3 lbs. of the so-called anhydrous zinc sulphate may be considered equal to 4 lbs. of the ordinary granular

zinc sulphate, provided it is stored in a dry place and kept well covered so that it does not absorb water.

The hydrated lime should be fresh and of high quality. The grade known in the trade as "chemical" is especially recommended.

The spray is prepared as follows: Fill the tank nearly full of water. Start the engine to give agitation. Add the zinc sulphate, which, if the lumps are well broken up, will dissolve in less than 5 minutes. After it has dissolved, the lime mixed with a small quantity of water to form a thin paste should be washed through the strainer into the tank. Finish filling the tank and agitate for 5 minutes or more before beginning to spray. The white precipitate settles somewhat rapidly but is easily kept in suspension by agitation. Use at once.

The chemical reactions taking place in the preparation of zinc-lime are not know to the writers. It is supposed that basic sulphates of zinc are formed. According to Pickering (1), who made a study of the chemical reactions in mixtures of this type, "The general character of the reaction appears to be similar with all the metallic sulphates examined, and with all the caustic alkalis. The metal is first precipitated as a basic sulphate of definite composition, which, on the addition of more alkali, is converted into a second, more highly basic sulphate, before any alkaline reaction becomes evident in the liquid. In some cases, the transformation takes place in two distinct stages.

"In every instance examined the products of the reaction, so far as the basicity of the precipitates is concerned, are the same, whether soda or lime is used, but the precipitates are not always identical in other respects, and this has been ascertained to be due to the fact that, when lime is used, they retain a considerable amount of the calcium sulphate formed in the reaction.

"With zinc sulphate and soda, the complete precipitation of the metal was coincident with the appearance of a permanent alkaline reaction, and no preliminary or secondary reaction was noticed. The alkali required was found to be 0.795 equivalent, indicating, therefore, the composition of the precipitate to be $5\mathrm{ZnO}$, SO_3 ."

EXPERIMENTS IN THE CONTROL OF BACTERIAL SPOT

Zinc-lime has given excellent results in the control of bacterial spot in orchards where its use has been supplemented by the employment of good cultural methods. Because peach trees lacking in vigor are especially susceptible to the disease, it is important that this unfavorable factor be overcome in so far as possible by good cultural practices. In spraying for the control of the disease it is important that the fruits and the underside of leaves be covered because the stomata are infection portals. Incidentally,

the covering of the young twigs probably prevents the formation of new bacterial spot cankers. Spraying with zinc-lime for the control of bacterial spot has restored the productiveness of trees that had been weakened by premature defoliation year after year and consequently had developed few fruit buds.

In addition to the experiments of 1928 and 1929 previously reported (2, 3, 4, 5, 6, 7, 8, 9), tests of zinc-lime as a preventive of bacterial spot were carried out at Vincennes, Indiana, in 1930 and 1931. In 1930 there was no crop but, despite the relatively dry season, results in the control of the disease on the leaves were obtained. In addition, notes on the effects of frequent applications of zinc-lime to peach trees were obtained. The experiments, as carried out, are outlined in table 1.

TABLE 1.—Composition and times of application of spray and dust materials used in experiments for the control of bacterial spot on peach leaves.

Vincennes, Indiana, 1930

Plot	Number	Composition of mixturesa	Dates of application
1 2 3 4 5 6 6A 7	60 36 36 32 20 20 20 24 24	Zine-lime, 4-4-50 '' '' 4-4-50 + casein-lime ½ lb. '' '' 8-8-50 '' '' 4-4-50 + wettable sulphur 2 lbs. '' '' 4-4-50 + copper sulphate ½ lb. Hydrated lime 4 lbs., water 50 gals. '' '15 lbs., '' 50 gals. Dust: monohydrated zine sulphate 30% hydrated lime 70% Dust: basic zine sulphate 20%, sulphur 65%, hydrated lime 15% (a commercial mixture)	April 26, May 9, May 21, June 4, June 19, July 7
9 10 11	24 24 24	Zinc-lime, 4-4-50 '' '' 4-4-50 Control	April 26, May 5, 9, 16, 22, 31, June 7, 13, 19, July 1, 7, 16 May 9, 16, 21, 31, June 13, July 1, 16 Not sprayed

^a Arsenate of lead was included in 2 applications on all plots. On dust plots it was substituted for part of the hydrated lime.

Bacterial spot was first noted May 8 on leaves of one of the controls. On May 14 a considerable number of infected leaves and a trace of defoliation were noted in the control plot. No infected leaves were found in the zinc-lime-sprayed plots. By June 27 infected leaves were present on all the control trees but none could be found in the zinc-lime-sprayed plots. On July 2 it was estimated that many of the controls had 10 per cent of their leaves infected. There were practically no infected leaves in the zinc-

TABLE 2.—Results of an experiment to determine the effectiveness of zinc-lime in the control of bacterial spot of peach fruits.

Vincennes, Indiana, 1931

	Total merchant-		38 97	32 97		98 38	19 96		26 94		28 98		19 98	42 92
Percentage of fruits having bacterial spot	Severe T	0	-	-		-	63		4		-	10	-	4
sentage of fruits bacterial spot	Moder- ate	0	ទា	01		-	C 3		c ₁		Н	∞	Н	4
Perc	Mildb	2	35	53		20	15		20		56	39	17	34
Total	fruit	4379	4352	5780		4813	3477		4435		2506	4572	9043	5605
Number	applica- tions	9	9	9		9	9		9		П	က	9	
	Composition of spiral of these	Zinc-lime 4-4-50	"" " $4-4-50 + \text{casein-lime } \frac{1}{2} \text{ lb.}$	" 4-4-50 + liquid licorice ex-	tract 2 lbs.	"" $\frac{4-4-50+\text{paste sulphur 2 lbs.}}{}$	Dust: monohydrate zinc sulphate 30%	hydrated lime 70%	Dust: a commercial dust containing	zine compounds	Zinc-lime 4-4-50	Control: sulphur dust	Zinc-lime 4-4-50	Control
Number	trees	9	9	9		9	9		9		9	9	12	9
	v arrety	J. H. Hale		9,9		3	8				,	e	Elberta	
1	ğ	Н	03	22		9	ø		6		10	11	12	13

^a Arsenate of lead in 2 applications for each plot.

^b Mild, spots inconspicuous and few; moderate, spots conspicuous but few; severe, spots conspicuous, numerous, causing disfigurement. Only the mild was merchantable.

lime-sprayed plots. On July 5 it was estimated that, on the average, the trees of the control plot had lost 5 times as many leaves as those of plot 1. On October 2 the estimated defoliation of the control trees was 15 per cent with about 20 per cent of the remaining leaves infected. At that time defoliation in the zinc-lime-sprayed plots was estimated at 2 per cent with 10 per cent of the remaining leaves infected. Plot 3, the zinc-lime 8-8-50 plot, and plot 9, receiving 12 applications of zinc-lime 4-4-50, were almost free of bacterial spot throughout the season. On October 2 the foliage of these plots was a rich, dark green, and uninjured. The copper caused injury to foliage in plot 5. In plot 4 the addition of wettable sulphur possibly reduced slightly the sticking properties and effectiveness of the zinc-lime. Neither the dust plots nor the lime plots gave any indications of controlling the disease. They were not superior to the control plot.

In 1931, at Vincennes, there was a heavy crop of fruit in the experimental plots, as indicated in table 2, which shows the results of experiments in the control of the disease on fruit. On all but Plot 10, applications of spray or dust were made at intervals of 2 weeks, beginning at petal fall. It will be noted that the number of infected fruits was much less on the zinc-lime plots and that nearly all the infections on the fruit of these plots were classified as mild. The first leaves showing bacterial spot were collected May 4, and the first fruit June 9. On June 19 the control trees showed slight defoliation. A small percentage of the fruit of these trees showed infection, but no infected fruit could be found on the trees sprayed with zinc-lime. On June 26 many of the nonsprayed Elberta trees had as many as 50 per cent of their leaves and many fruits infected. There was a noticeable number of infected leaves on some of the zinc-lime-sprayed Hale and Elberta trees and a trace of defoliation, but the fruit and foliage of plot 1 were nearly free of infection. During July the development of bacterial spot was apparently checked by the hot, dry weather but was favored by more rain and cooler weather in August. At the beginning of fruit harvest, August 24, defoliation on plot 1 was estimated to be 5 per cent, on plot 2, 20 per cent, and on the remaining zinc-lime-sprayed plots. 10 per cent. On plots 8 and 9, the zinc-lime-dust plots, and on the control, defoliation was estimated to be 35 per cent.

The orchard used in these experiments is on light, sandy soil and, in its earlier years, was annually defoliated by bacterial spot. By use of leguminous cover crops and the zinc-lime spray, defoliation in later years has been largely prevented and, consequently, the trees have become vigorous and productive.

EXPERIMENTS IN THE CONTROL OF SCAB

Since scab was either absent or of slight importance in the Indiana and Arkansas orchards in which the bacterial-spot-control experiments were

performed, experiments in control were conducted at Arlington Farm, Rosslyn, Virginia, in 1930 and 1931. Trees known to have been heavily infested with scab in previous years were selected and a large limb on each tree received applications of zinc-lime 4-4-50.

In 1930 the unprecedented drought caused infection of the fruit to be so light that results were not recorded. In 1931 rains in July and August were favorable for a moderate infestation and results were recorded.

Six seedling and 6 budded peach trees were used in the 1931 experiment. In all cases, a limb adjacent to the sprayed limb and of about the same size and the same distance from the ground, was used as a check or control. A small hand pump rather than a larger outfit was used in order to confine the spray to the selected limbs.

The applications concerned in scab control were made (1) May 27, (2) June 19, (3) July 13. Six trees received all 3 applications, and 6 received only the 1st and 3rd.

Because of severe hail injury in late July, records had to be taken about 1 week before ripening in anticipation of a masking of the scab spots by the brown rot that usually follows late-season hail injury. Results from 1 of the budded trees were not taken because no scab developed, even on the nonsprayed limbs. Factors unfavorable to control, which do not exist in commercial practice, were: (1) the nonsprayed part of the tree was a potential infection source for the sprayed fruit; (2) the small hand pump did not give good coverage, principally because it did not give pressure sufficient to blow aside the leaves that covered the fruit.

In recording results a peach was counted as scabby even if it had but 1 scab spot. A peach with more than 5 spots was recorded as severely scabbed. Many of the control fruits, especially those of the late-season varieties, were half-covered with a crust of coalesced scab spots. This was particularly true of the Heath Cling variety which, because its fruit ripens late in the season, should have received an additional application in August. Table 3 is a summary of the results. In this table a sprayed limb and a control on the same tree are given the same plot number.

It appears from these results that the application made June 19 had little effect on scab control. Very little scab developed until late in July, when frequent rains, in contrast with the bright sunny weather of spring and early summer, favored development. The results are fairly consistent and indicate that zinc-lime can be depended upon to control at least moderate outbreaks of scab.

EXPERIMENTS IN THE CONTROL OF BROWN ROT

Results in the control of brown rot are not conclusive but indicate that zinc-lime will definitely check and probably control the disease. In experi-

TABLE 3.—Results of spraying peach trees with zinc-lime 4-4-50 for the control of scab (Cladosporium carpophilum). Arlington Farm, Rosslyn, Virginia, 1931

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				Fruit	
Plot	Variety	Dates sprayed	Total	Percentage having scab	Percentage having severe scab
1	Unnamed seedling	May 27, July 13	39	18	0
1A	"	Control	24	54	0
2		May 27, July 13	28	50	0
2A	ii ii	Control	27	100	78
3		May 27, July 13	62	34	3
3A.	a a	Control	32	59	6
4		May 27, June 19, July 13	19	47	11
4A		Control	12	67	33
5		May 27, June 19, July 13	87	28	0
5A	i co	Control	21	71	19
6	u u	May 27, June 19, July 13	102	35	3
6A	"	Control	58	79	26
7	Heath Cling	May 27, June 19, July 13	78	47	18
7A		Control	71	100	93
8	Early Elberta	May 27, June 19, July 13	91	1	0
8A		Control	82	46	9
9	Belle	May 27, June 19, July 13	140	6	0
9A	a	Control	71	25	0
10		May 27, July 13	88	28	0
10A	"	Control	76	22	9
11	Carman	May 27, July 13	107	8	0
11A		Control	95	52	3
All sp	rayed plots	1	841	23	3
All co	ntrol plots		569	57	23

ments for the control of brown rot as an orchard disease the reliability of the results is frequently under suspicion because of the relation of the disease to insect attacks and to moisture conditions, both of which may vary greatly in different parts of an orchard. Probably storage tests of the picked fruit from plots variously treated give more reliable results than those obtained from examination of newly picked fruits. In storage tests, all wormy fruit is thrown out, and an attempt can be made to use fruit of the same degree of ripeness. Also the fruit from all plots can be kept under the same conditions of temperature and moisture.

In 1928, in Vincennes, Indiana, records on brown rot were taken, together with those on bacterial spot, for the control of which the experiment was primarily designed. The zinc-lime plot of J. H. Hale peaches was sprayed 6 times, but only the last application, which was made on July 16, would be effective against brown rot. The variety J. H. Hale regularly

matures a crop of large spherical "normal" fruits followed 2 to 3 weeks later by the small elongated fruits, which, in Indiana, are called "nubbins." The results of spraying both normal fruit and nubbins for the control of brown rot, shown in table 4, indicate success in checking loss from brown rot

TABLE 4.—Results of experiments on the control of peach brown rot by means of zinclime sprays applied April 26, May 11, May 25, June 12, June 30, July 16. Vincennes, Indiana, 1928. Picked fruit of the J. H. Hale variety

Dlat	Number			nal fruit Aug. 20–25		oin'' fruit Sept. 10-11
Plot No.	of trees	Composition of spray	Total	Per- centage rotted	Total	Per- centage rotted
1	9	Zinc-lime, 4-3-50, + casein- lime ½ lb.	1467	3	1137	9
2	6	Control	328	8	1034	21

on both normal fruit and nubbins. There was no wormy fruit on either sprayed or control trees to complicate the results.

In 1929, at Springdale, Arkansas,³ one plot in an orchard of 16-yearold Elbertas was sprayed 7 times for the control of bacterial spot, while the control plot received the usual applications of self-boiled lime-sulphur for the control of brown rot and scab. The last application of spray was made July 16, and the fruit, which ripened very unevenly, was picked August 5 to 19. The trees were hard to spray, being large, even for their age, and closely planted. In the zinc-lime plot there were 103 trees, while the remainder of the orchard comprised the control plot.

In the zinc-lime plot the crop of 10 trees and in the control the crop of 12 trees were examined for brown rot. The results, as shown in the 2 upper rows of table 5, are not very convincing, but when a group of trees in the zinc-lime plot is compared with an adjacent group in the sulphur plot, as is done in the last 4 rows of the table, there is little choice between the zinc-lime and sulphur sprays. The failure of both to give better results was probably due to the previously mentioned difficulties in applying the sprays.

In 1929, at Vincennes, Indiana, there was not enough brown rot in the experimental orchards at picking time to yield results worth recording, but there was enough to distribute conidia about the orchard. From a zinc-lime plot and a control plot, J. H. Hale peaches of medium size and

³ These experiments were carried out by John C. Dunegan, Associate Pathologist, Division of Horticultural Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture.

TABLE 5.—A comparison of zinc-lime and self-boiled lime-sulphur sprays for the control of peach brown rot. Picked fruit of the Elberta variety.

Springdale, Arkansas, 1929

		Fr	uits
Composition of spray	Number of trees	Total	Percentage rotted
Zinc-lime, 4-3-50, + casein-lime ½ lb	10	7032	19
Self-boiled lime-sulphur, 8-8-50	12	6052	12
Zinc-lime, 4-3-50, + casein-lime $\frac{1}{2}$ lb	Group 1, 4 trees	1313	46
Self-boiled lime-sulphur, 8-8-50	" 1A, 3 "	384	57
Zinc-lime, 4-3-50,+ casein-lime $\frac{1}{2}$ lb	" 2, 6 "	5719	15
Self-boiled lime-sulphur, 8-8-50	" 2A, 6 "	4874	12

hard ripe were selected and kept in baskets in basement storage without refrigeration directly after picking in August. The zinc-lime plot had received 8 applications of spray, but only the last 1, applied 15 days before picking, would be effective in checking rot of stored fruit. The controls had been sprayed with a commercial wettable sulphur 38 days before picking and were therefore practically unprotected. The results are shown in table 6. Evidently the zinc-lime checked the rot, since the control fruit

TABLE 6.—Effect of zinc-lime spray, applied 15 days before picking, on the prevention of brown rot. J. H. Hale peaches stored without refrigeration. Vincennes, Indiana, 1929

		Percent	age of brow	n rot on sto	red fruit
Composition of spray	Total fruit	After 2 days	After 4 days	After 7 days	After 10 days
Zinc-lime, 4-3-50, + casein-lime ½ lb	153	1	25	71	95.
Control	139	9	60	91	98

rotted much more rapidly than that sprayed with zinc-lime.

The seasons of 1930 and 1931 were too dry for the development of sufficient brown rot to give results in experimental trials.

EFFECT OF ZINC-LIME SPRAY ON THE PEACH

No injury to the peach by the zinc-lime spray has been noted; even 6 applications at double the strength commonly used in these experiments

caused no injury. (Table 1.) In 1930 the 24 peach trees in the Indiana experiments (Table 1) receiving 12 applications of zinc-lime, 4-4-50, were the most vigorous-appearing trees in the orchard at the end of the season. In 1931 those receiving 11 applications (Table 2) appeared at least as vigorous as any in the orchard.

The fruit of the zinc-lime plots in all trials compared favorably in color with that of trees receiving sulphur sprays. The size and number of fruits appeared to be favorably affected, but whether by control of bacterial spot on the leaves, by foliage stimulation, or by direct effect on the fruit, is not known. Unless deep-green because the trees are already in a high state of vigor, the leaves of zinc-lime-sprayed trees were noticeably a deeper green and often larger than those of the controls that were either not sprayed or sprayed or dusted with other materials. In 1928 leaves from zinc-lime-sprayed trees and from controls, respectively, were measured with a planimeter. Attempts were made to collect leaves of the same age and relative position on zinc-lime-sprayed trees and on controls. The average area of 136 leaves taken from 10 twigs of a zinc-lime-sprayed tree was approximately 0.35 sq. in. larger than that of 123 leaves of an adjacent control. With so many chances for error, these results, of themselves, cannot be considered significant, but they support the field observations.

Zinc-lime-sprayed trees have frequently retained foliage on short laterals long after the leaves had been shed from practically every short lateral on the control trees in July, and in every experiment they have retained their foliage later in the season than the controls. This retention of foliage can be accounted for partly by control of bacterial spot and partly by lack of spray injury, but it has occurred also in orchards in light soil where there was little or no bacterial spot and no apparent spray injury on the control trees. It seems to be due, in part at least, to a stimulation or other physiological effect, wholly apart from control of pathogens or from lack of injury. The stimulative effect of small quantities of zinc on plants is well known. With the zinc-lime sprays the effect seems to be a direct one because it may appear very soon after an application is made and because no quickly apparent results have been obtained from applying solutions of zinc sulphate or the zinc-lime mixture to the soil. The retention of foliage by the peach trees sprayed with zinc-lime is one of the most notable features of the treatment. It is of particular importance for orchards in light soil and subject to bacterial spot and spray injury. Premature defoliation in such orchards not only weakens the trees but frequently reduces the crop for the following year by preventing the fruit buds from maturing.

COMBINATION OF ZINC-LIME WITH OTHER SPRAY MATERIALS

Zinc-lime has been used by the writers in combination with wettable sulphur without apparent injury to peach trees and without appreciably lessening the efficacy of zinc-lime in the control of bacterial spot. The combination appears to make a satisfactory spray. Many growers have used this combination with apparent success.

In most of the experiments reported upon in this paper arsenate of lead was added to at least 2 of the early-season applications. Arsenate of lead in combination with zinc-lime caused little or no injury, usually much less than when combined with wettable sulphurs and lime or with lime alone. Growers who have used zinc-lime and arsenate of lead in combination have noted much less injury than with the sulphur-arsenate of lead-lime combination. The writers have observed the same differences in orchards commercially sprayed by growers. One large grower uses the zinc-lime and arsenate of lead combination largely for the purpose of avoiding arsenical injury to foliage, twigs, and buds.

In the experiments of 1930 (Table 1) there was considerably more arsenical injury in plot 6A, which received applications of hydrated lime at the extremely high rate of 15 lbs. to 50 gals. of water in combination with arsenate of lead, than in plots 1, 2, 3, and 4, receiving zinc-lime in combination with arsenate of lead.

SUMMARY

In experiments and in commercial orchards zinc-lime, a spray composed of zinc sulphate, hydrated lime, and water, when applied to peach trees, has greatly reduced infection by *Bacterium pruni*. Because infections may occur from early spring till late summer, 5 to 7 applications of the spray were necessary. Because the stomata are infection portals, special care must be taken to cover the underside of leaves. Coverage of young twigs probably prevents formation of new bacterial-spot cankers.

Zinc-lime, in experimental trials on a small scale, has controlled mild and moderately severe cases of peach scab (Cladosporium carpophilum), and there are indications that it will, at least, control mild cases of peach brown rot (Sclerotinia fructicola). Because its effectiveness against severe cases of scab and brown rot has not been tested, it is not yet known whether it would be as effective as the sulphur sprays under conditions favoring heavy infestation.

Zinc-lime, even at twice the strength here recommended, has not injured fruit, foliage, or any part of the peach tree. On the contrary, it has frequently caused the leaves to become deeper green and larger than those of the controls. There is some indication that fruit development also is stimulated. Trees receiving 12 applications were, at the end of the season, the most vigorous-appearing ones in the orchard.

Arsenate of lead, when combined with zinc-lime, in experimental trials and in commercial use, generally has caused noticeably less injury to

foliage, twigs, and buds than when combined with sulphur fungicides and lime or with lime alone. In experimental trials and in trials by growers a mixture of one of the wettable sulphurs, commonly used on peaches for the control of scab and brown rot, and zinc-lime, when applied to peach trees, caused no apparent injury and seemed not to reduce appreciably the effectiveness of the constituents.

Zinc-lime in dust form was ineffective in the control of bacterial spot on peach leaves and less effective than the spray in the control of the disease on fruit. It has not been tested as a fungicide for the control of brown rot and scab.

DIVISION OF HORTICULTURAL CROPS AND DISEASES.

BUREAU OF PLANT INDUSTRY,

UNITED STATES DEPARTMENT OF AGRICULTURE.

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SEED TREATMENTS WITH CHEMICAL DUSTS AND FORMALDE— HYDE FOR SMUT CONTROL IN OATS

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INTRODUCTION

The economic importance of smut control in oat production becomes readily apparent when the heavy annual loss caused by the oat-smut pathogens is considered. According to estimates of the plant Disease Survey for the 11-year period 1917–1927, inclusive, for which estimates are available, the average loss from smut in oats in the United States has amounted annually to almost 50,000,000 bushels.² In single States, annual loss as high as 10 per cent frequently has been reported. In 1928 the estimated oat-smut loss was 35 per cent in South Carolina and 20 per cent in North Carolina. Within the last few years, moreover, new physiologic forms of the pathogens (*Ustilago avenae* (Pers.) Jens. and *U. levis* (Kell. and Sw.) Magn.) that are taking a heavy toll in hitherto-resistant varieties (16) have appeared.

¹ The writer gratefully acknowledges his indebtedness to Mr. T. R. Stanton, Division of Cereal Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture, for identifying the oat varieties used in the experiments, and to Messrs. L. L. Davis and R. W. Leukel, of the same Division, for making the counts of healthy and smutted heads in the test at Aberdeen, Idaho, in 1930.

² United States Department of Agriculture. Bureau of Plant Industry. The following mimeographed material from Plant Disease Bulletin (or Reporter):

Estimate of crop losses due to plant diseases. 1917. Plant Disease Bul. 2: 1-18. 1918.

Crop losses from plant diseases. 1918. Sup. 6: 186-213. 1919.

Crop losses from plant diseases in the United States in 1919. Sup. 12: 307-332.

Crop losses from plant diseases in the United States in 1920. Sup. 18: 317-338. 1921.

Crop losses from plant diseases in the United States in 1921. Sup. 24: 489-510. 1922.

Crop losses from plant diseases in the United States in 1922. Sup. 30: 462-490. 1923.

Crop losses from plant diseases in the United States in 1923. Sup. 36: 318-348. 1924.

Crop losses from plant diseases in the United States in 1924. Sup. 43: 381-410. 1925.

Crops losses from plant diseases in the United States in 1925. Sup. 49: 382-412.

Crop losses from plant diseases in the United States in 1926. Sup. 56: 394-423.

Crop losses from plant diseases in the United States in 1927. Sup. 64: 370-399.

The formaldehyde (liquid) treatment has been recommended for the control of oat smut since 1897 (1). The continued heavy loss from smut in oats would seem to indicate, however, that the treatment is not very extensively used. This seems due chiefly to two causes: (1) The reluctance of many growers to use any form of wet treatment for grains and (2) the not uncommon reports of injury to the germination of seed oats resulting from treatment with formaldehyde.

In the past few years an increasing number of American and foreign manufacturers have marketed various chemical dusts prepared especially for treating seed oats for smut control or for treating diseased seed of various crops. In 1928, 1930, and 1931 the writer tested at the Arlington Experiment Farm, Rosslyn, Virginia, or at the Aberdeen Substation, Aberdeen, Idaho, a total of 21 dusts on 20 lots of oats, naturally inoculated either with loose or covered smut or both, obtained from heavily smutted fields in various parts of the United States. In 1930 and 1931 the formaldehyde (liquid) treatment also was included in the tests. Some of the dusts produced excellent control of both smuts without injury to seed germination. The results of the tests follow.

EXPERIMENTAL WORK

Materials, Methods, and Results

1928 experiments. The seed lots used in these experiments were from the 1927 crop. Lot 1 was a mixture mostly of Silvermine from New Jersey; lot 2 was a mixture of Silvermine, Fulghum, and an unknown variety from North Carolina; and lot 3 was Silvermine from Ohio.

The crops that produced the seed lots used in the experiment contained relatively high percentages of the smutted heads that served as the source of inoculum for producing infection under natural conditions. None of the seed lots were artificially smutted.³

The chemical dusts were applied at the rate of 4 oz. to the bushel of seed, on April 3, 1928. Lots of 96 gm. of seed and 750 mg. of dust were placed in 1-lb. tins with screw caps. The latter were turned end over end for 5 minutes in a motor-driven box (described and illustrated by Leukel (13)), making 32 revolutions per minute. Ninety-six-gram (check) lots, to which no chemical dust was added, also were similarly agitated. Through the use

³ The artificial method of inoculation in which dry spores are applied to the exterior surfaces of the glumes has been shown by Reed and Faris (17), Kolk (10), and others to be very effective in producing infection under appropriate seed-bed conditions. However, in view of the work of Zade (22, 23) and his coworkers in Germany and of Gage (5) in the United States, showing the importance of intraglume inoculum in nature, it seemed expedient to use only seed that was inoculated under natural conditions in the floral stage.

of this method the dusts were uniformly applied, and treated and nontreated seed were handled alike, except as to the dust application. In applying the dusts to such small quantities of seed relatively large portions of the finely ground chemicals adhered to the inner surfaces of the containers used in treatment. For this reason the dusts were applied at the 4-oz. rate per bushel instead of the 3-oz. rate usually recommended. Immediately after treatment of the seed with the different dusts, except the sulphurs, on April 3, the caps of the treating tins were removed and the treated seed was exposed to the outside air. The following day 1 lot of 250 seeds of each treated unit and 2 lots of 250 seeds of each nontreated unit were counted and packeted. The lots were sown in rod rows at the Aberdeen Substation, Aberdeen, Idaho, on April 11. On April 15, seed of lot No. 1 was treated with the sulphur dusts shown in table 2, following the method used in the application of the other dusts. The following day 12-gm. samples of all of the dusted and nondusted units of lot No. 1 were packeted. The seed was sown in rod rows at the Arlington Experiment Farm, Rosslyn, Virginia, on April 20. The results at Aberdeen and at Arlington Farm are shown in tables 1 and 2, respectively.

The combined data in tables 1 and 2 show that Ceresan (especially Ceresan K-1-C) was the only dust effective in smut control under the conditions of the experiment in 1928 and that none of the dusts reduced seedling emergence.

1930 experiments. In 1930, 10 lots of seed from heavily smutted fields in different States were treated each with 10 different chemical dusts and the formaldehyde liquid dip.

Seed lot 4 was Green Russian, from Iowa, from the 1928 crop. Lot 5 was a mixture, mostly Green Russian, from New Jersey, from the 1927 crop; seed lots 6, 7, and 8 were Kanota, from the 1928 crop. Lots 6 and 8 came from Kansas and lot 7, from Wisconsin. Lots 9 to 13 were from the 1929 crop, lot 9 being Fulghum from Arkansas, lots 10, 11, and 12, Burt, 60-Day, and Iowa 103, respectively, from Illinois, and lot 13 was Frazier, from New Jersey.

The dusts were applied on March 14. The technique and rate of application used in 1928 were again employed. Immediately after treatment the lids of the treating tins were removed, thus exposing the treated seed to the outside air, except that the tin containing seed treated with Smuttox was kept closed until the following day⁴ and the tin containing seed treated with Kantsmut dust was kept closed for 3 days after treatment.⁵ The

⁴ Manufacturers' instructions are to keep treated seed sacked and allow same to stand at least 3 hours, or, preferably, overnight, before sowing.

⁵ Manufacturers' instructions are to keep treated seed covered 2 to 5 days after treatment.

TABLE 1.—Seedling emergence and smuttiness in 3 lots of oats grown in 1928 at Aberdeen, Idaho, from naturally inoculated seed. Seed nontreated or treated with 1 of 6 chemical dusts and sown in rod rows

	Seec	Seed lot 1	Seed	Seed lot 2	Seed	Seed lot 3			Seed lots	Seed lots 1, 2, and 3	
Seed treatment	Number of heads in rod row	Number of smutty heads	Number of heads in rod row	Number of smutty heads	Number of heads in rod row	Num- ber of smutty heads	Num- ber of heads	Number of smutty heads	Per- cent- age of smutty heads	Number of seed- lings emerged from 750 seeds (250 seeds from each of the 3 lots)	Per- cent- age of emer- gence
Abavit B	458	131	612	34	375	42	1445	207	14.3	620	82.7
Tillantin höchst	414	33	602	က	381	21	1397	57	4.1	634	84.5
Tutan	450	191	618	48	385	28	1453	237	16.3	645	86.0
Nontreated	452	173	553	99	448	88	1453	317	21.8	637	85.5
Ceresan (K-1-C)	423	9	929	c 3	477	0	1476	∞	0.5	641	85.5
Ceresan (K-1-D)	447	15	522	0	386	0	1355	15	1.1	632	84.3
D. D. D. 68	538	141	260	38	395	56	1493	202	13.7	641	85.5
						-					

TABLE 2.—Smuttiness in oats grown in 1928 at Arlington Experiment Farm, Rosslyn, Virginia, from naturally inoculated seed. Seed nontreated or treated with each of 11 chemical dusts

Seed treatment	Number of heads in rod row	Number of smutty heads	Percentage of smutty heads
Abavit B	442	139	31.4
Tillantin höchst	397	46	11.6
Tutan	306	57	18.6
Anchor Sulfur	357	86	24.1
Nontreated	371	65	17.5
Ceresan (K-1-C)	462	0	0.0
Ceresan (K-1-D)	480	9	1.9
D.D.D. 68	449	47	10.5
Nontreated	431	68	15.8
Niagara Sulfur No. 76	456	44	9.6
77	482	40	8.3
78	482	67	13.9
79	554	84	15.2

a Lot No. 1.

formaldehyde dip was applied March 15. The oats contained in half-filled cheesecloth bags were immersed for 10 minutes in a 1 to 320 solution of formaldehyde and water. The sacks then were left to drain for 1 hour, piled together, covered with sacks, and left overnight. The following morning the treated seed was spread out in thin layers and left 2 days to become thoroughly dry and aerated.

Following application of the different treatments as noted above, 2 sets of 250 seeds of each treated and nontreated unit were packeted and sown in rod rows at Arlington Experiment Farm, the first set on March 19, the second on April 14. Following treatment 2 sets, consisting of 12-gm. samples each, also were prepared. These were sown at the Aberdeen Substation, Aberdeen, Idaho, the first set on April 15, the second on May 12. Similar results were obtained from the early and later sowings at Arlington Experiment Farm, and for convenience, the data have been combined in table 3. At Aberdeen a higher percentage of smutty heads appeared in the earlier sowing, as shown in table 4.

The data in tables 3 and 4 show that smut was entirely eliminated in the early and later sowings at both Aberdeen and Arlington Experiment Farm, when the plants were grown from seed treated with the formaldehyde dip or the dusts Dubay 655 and Dubay 665. Excellent control also was obtained from seed treatment with Kantsmut dust, Corona Oat Dust, and Smuttox dust. Ceresan was fairly effective at Arlington Experiment

TABLE 3.—Seedling emergence and smuttiness in 10 lots of oats grown in 1930 at Arlington Experiment Farm, Rosslyn, Virginia, from naturally inoculated seed. Seed nontreated or treated with formaldehyde (liquid) or with 1 of 10 chemical dusts

Seed treatment	Nun	1ber of (300		ecutive eartive	ads in e head ch of 2	smutty heads in 600 heads consecutive heads in a rod each of 2 series)	eads (r. rod)	of eacl row f	ach seed from	l lot.	Number of smutty heads in 6,000 heads	Per- cent-	Number of seedlings from 1,000 seeds	Per- cent- age
	Lot 4	Lot	Lot	Lot 7	Lot 8	Lot	Lot 10	Lot 11	Lot 12	Lot 13	each of the 10 lots)	smutty heads	each of the 10 lots)	of emer- gence
Acco dust	6	47	99	28	78	27	10	16	21	57	340	5.67	918	91.8
Ansbacher grain dust	0	က	0	0	Н	Н	П	П	c1	4	13	0.22	806	8.06
Ceresan dust	0	0	0	0	0	0	0	0	0	H	H	0.02	206	2.06
Dubay dust No. 655	0	0	0	0	0	0	0	0	0	0	0	0	902	90.5
Dubay dust No. 665	0	0	0	0	0	0	0	0	0	0	0	0	913	91.3
Nontreated	00	28	29	28	28	43	19	12	01	70	267	4.45	918	91.8
Formaldehyde dip	0	0	0	0	0	0	0	0	0	0	0	0	968	9.68
Kantsmut dust	0	0	0	0	0	0	0	0	0	0	0	0	922	92.2
Oat Dust (Corona)	0	0	0	0	0	6.1	0	0	0	0	67	0.03	934	93.4
Smuttox dust	0	0	0	0	0	0	0	П	0	0	Н	0.05	892	89.5
Sterocide dust	5	21	37	11	87	42	19	13	Ø	44	222	3.70	925	92.5
WaWa dust	0	-	-	0	0	0	Н	0	0	4	9	0.10	998	9.98

TABLE 4.—Smuttiness in 10 lots of oats grown in 1930 at Aberdeen, Idaho, from naturally inoculated seed. Seed nontreated or treated

				Numbe	r of s	mutty	heads	in ro	w row	Number of smutty heads in row rows containing	aining		approximately 650 heads per row	ly 65) head	s per	row			
Seed treatment					First 8	sowing								Se	Second s	sowing		. 24		
	Lot 4	Lot	Lot	Lot 7	Lot 8	Lot 9	Lot 10	Lot 11	Lot 12	Lot 13	Lot 4	Lot 5	Lot 6	Lot 7	Lot 8	Lot.	Lot 10	Lot 11	Lot 12	Lot 13
Acco dust	45	48	7.1	155	- 26	83	69	81	24	185	45	99	27	101	45	22	58	18	4	51
Ansbacher grain dust	0	ro	21	ŭ	9	39	9	0	0	96	0	Ħ	67	4	63	c ₁	0	0	0	Н
Ceresan dust	0	0	-	-	63	.c	0	0	0	18	0	0	0	0	0	0	0	0	0	c/1
Dubay dust No. 655	•	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Dubay dust No.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Nontreated	59	16	43	70	80	7.1	28	24	9	157	9	19	c1	37	10	∞ ∞	c)	0		15
Formaldehyde	•	_		_	_	c	c	Ç	C	0	0	0	0	0	0	0	0	0	0	0
Oat Dust (Corona)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Kantsmut dust	0	0	0	0	0	0	0	0	0	c 1	0	0	0	0	0	0 (0 (0 9	0 0	0
Smuttox dust		0	0	0	0	0	0	0	0	0	0 (0 ;	0	0 9	0 9	 ع د	0 ;) °	0 6
Sterocide dust		27	74	43	98	100	96	80	ж (152	9	31.	္ ေ	5	27 0	7	1 9	-	o c	7 -
WaWa dust	0	<u>.</u>	16	Н	63	15	9	က	9	34	>	റ	>	>	7	>	>	>	>	4
The second secon	-	-	_								-	-		-	1	-				1

Farm and in the second sowing at Aberdeen but less effective in the first sowing at Aberdeen. WaWa dust and Ansbacher grain dust did not reduce the smut sufficiently to merit further consideration. Sterocide had little influence on the occurrence of smut. It is interesting to note that plants from seed treated with Acco dust produced more smut than those from nontreated seed in 5 of the 10 lots at Arlington Experiment Farm and in all of the lots at Aberdeen, Idaho. None of the treatments appreciably reduced seedling emergence. (Table 3.)

1931 experiment. The experiment was concluded in 1931. In this year 3 of the lots (Nos. 9, 10, and 11) used in 1930 and 7 new lots from very smutty plots or fields in different States were each treated with the formaldehyde liquid dip and the dusts that produced satisfactory control the previous year, namely: Ceresan, Dubay No. 655, Dubay No. 665, Kantsmut, Oat Dust, and Smuttox.

Seed lots Nos. 9, 10, and 11 were from the 1929 crop, and lots 14 to 20 were from the 1930 crop. Lot 9 was Fulghum from Arkansas, and lots 10 and 11 were Burt and 60-Day, respectively, from Illinois. Lot 14 was Kanota, lot 15, Green Russian, lot 16, Frazier, and lot 19 was Fulghum, all from New Jersey. Lots 17 and 18 were unknown varieties from the same State. Lot 20 was Fulghum from South Carolina.

The dusts were applied March 3, 1931. The technique and rate of application and the post-treatment handling of the treated seed closely followed those of the previous years. The seed treated with Smuttox and Kantsmut was again kept confined in the closed tins for 1 and 3 days, respectively, after treatment, in accordance with manufacturers' instructions on the label of the product. The formaldehyde dip also was applied according to the procedure in 1930. Twelve-gram samples of each treated and nontreated unit were sown in rod rows in triplicate series at the Arlington Experiment Farm, on April 9. The following day 100 seeds of each treated and nontreated unit were sown in soil in a greenhouse bench to study further the effects of the treatments on seedling emergence. The results of the experiment are presented in table 5.

The data in table 5 show that all of the treatments produced excellent control of both loose and covered smuts, particularly the formaldehyde (liquid) dip. The latter reduced emergence of seedlings from 93.3 per cent to 88.8 per cent, but with this exception none of the treatments reduced emergence below 90 per cent.

DISCUSSION

Under the conditions of the present experiments, the following treatments applied to naturally inoculated seed oats eliminated smut or reduced it to 1 per cent of smutty heads or less in plants grown from the treated

TABLE 5.—Seedling emergence and smuttiness in 10 lots of oats grown in 1931 at Arlington Experiment Farm, Rosslyn, Virginia, from naturally inoculated seed. Seed nontreated or treated with formaldehyde (liquid) or with 1 of 6 chemical dusts

Seed treatment	Num	nber o. (30	f smu)0 con	tty he secuti ea	ads in ve hea ch of	heads in 900 hea utive heads in a each of 3 series)	of smutty heads in 900 heads of each s'300 consecutive heads in a rod row from each of 3 series)	of ead row f	Number of smutty heads in 900 heads of each seed lot. (300 consecutive heads in a rod row from each of 3 series)	d lot.	Number of smutty heads in 9,000 heads.	Per- cent- age of	Number of seed- lings from 1,000 seeds.	Per cent-age of
	Lot 9	Lot 10	Lot 1	Lot 14		Lot Lot 15 16	Lot Lot 17 18	Lot 18	Lot 19	Lot 20	from each of the 10 lots)	smutty heads	from each of the 10 lots)	emer- gence
Ceresan dust	0	0	0	0	0	0	0	0	23	0	67	0.022	929	92.9
Dubay dust No. 655	0	0	0	0	0	H	0	0	0	0	-	0.011	934	93.4
Dubay dust No. 665	0	0	0	0	0	0	0	0	П	0	-	0.011	933	93.3
Nontreated ^a	158	114	128	310	103	332	152	221	237	231	1986	22.066	933	93.3
Formaldehyde dip	0	0	0	0	0			0	0	0	0	0.00	888	88.8
Kantsmut dust	П	0	67	က	0	H	0	0	7	67	16	0,177	910	91.0
Oat Dust (Corona)	0	0	0	C 1	-	01	0	0	5	c 1	12	0.133	606	6.06
	Н	Н	0	6	0	9	0	Н	0	c 1	20	0.222	918	91.8
													and the second s	

a The occurrence of either loose or covered smut in plants from nontreated seed of the 10 lots was as follows: Lots 9, 10, 11, 14, 16, 19, and 20-apparently all heads loose smut. Lot 15-loose and covered smut heads occurring in the ratio approximately of 1 to 2, respectively. Lots 17 and 18—covered smut predominant; loose smut present only in a few heads. seed: The formaldehyde (liquid) dip and the dusts Dubay No. 655, Dubay No. 665, Smuttox, Kantsmut, and Corona Oat Dust. Ceresan dust also produced excellent control except that in one case plants from treated seed contained 2.8 per cent smutty heads. The formaldehyde (liquid) dip caused a reduction in seedling emergence from 93.3 per cent to 88.8 per cent in 1931; otherwise, none of the treatments appreciably affected germination.

Some of the treatments listed above have been studied previously with regard to effectiveness in smut control and influence on seed vitality and on yield of plants from treated seed. The effectiveness of the formaldehyde (liquid) treatment in the control of smut in oats when applied as a spray, sprinkle, or dip has been repeatedly shown since Bolley's introduction of formaldehyde as a fungicide in 1897 (1) and is unquestioned. A survey of some of the reports with regard to the effect of the formaldehyde treatment on germination shows that in many cases results similar to those obtained by the writer have been obtained, i.e., little or no injury to germination was brought about through seed treatment. However, reports of serious impairment of seed vitality not infrequently have been obtained (4, 11, 12, 14, 15, 20, 21). Sayre and Thomas (19) also state that the effectiveness of the formaldehyde (liquid) treatment in smut control is unquestioned but, in Ohio, "there have been many instances of serious injury to germination." Hurd (8) has well demonstrated the importance of post-treatment conditions of humidity in relation to injury to seed wheat treated with formaldehyde. It seems possible that a similar relation may hold true for formaldehyde-treated oats.

Previous reports concerning the new Dubay dusts included in the writer's tests apparently have not been made. Ethyl mercury phosphate is the active ingredient. The dusts Smuttox, Kantsmut and Corona Oat Dust are similar in composition and contain 4, 4, and 5 per cent formaldehyde, respectively. The formaldehyde-dust treatment for control of smut in oats, invented by Sayre and Thomas (19) of the Ohio Agricultural Experiment Station and introduced in 1927, has been extensively tested in recent years with uniformly good results. Sayre and Thomas (19) and Sayre (18) report excellent control without seed injury in both experimental tests and tests by growers in many parts of Ohio. Similar results have been obtained by other investigators in different parts of the United States and Canada in tests of the commercial formaldehyde dusts, especially Smuttox (2, 3, 6, 7, 9, 11). Ceresan dust also has been extensively tested in recent years and has given satisfactory results (2, 3, 6, 7, 9, 11).

Dusts of different chemical composition are now available for controlling the smuts and other small grain parasites amenable to control through surface disinfection of the seed. In the light of recent progress in the development of seed dusts it seems reasonable to suppose that single dusts may be evolved that will replace the confusing array of wet, semidry, and dry treatments with different chemicals, different methods of application, and different post-treatment handlings now recommended. In fact, Ceresan dust already has been shown to be effective in the control of most of the pathogens in this class. Recent improvements in the manufacture of certain dusts indicate that one of the most serious objections to the dusts, the relatively high cost, may be eliminated.

Doubtless a universal dry treatment that might prove to be inexpensive, easily applied, effective in smut control, and noninjurious to seed germination and to man would tend to popularize the treatment of seed oats and the seed of other small grain crops.

SUMMARY

- 1. Tests of dust fungicides on oats, both at Arlington Experiment Farm, Rosslyn, Virginia, and the Aberdeen Substation, Aberdeen, Idaho, in 1928 and in 1930 and at Arlington Experiment Farm in 1931 included 21 chemical dusts and the formaldehyde (liquid) dip on 20 lots of naturally inoculated seed. The content of loose or covered smut or both in plants from nontreated seed of the different lots generally ran high. The dusts were applied at the rate of 3 oz. or slightly more per bushel. The formaldehyde dip was applied by immersing seed for 10 minutes in a 1–320 formaldehydewater solution, followed by draining off the excess liquid, covering seed overnight, and aeration for several days after treatment.
- 2. With regard to smut control, the different seed treatments behaved as follows:
 - A. The formaldehyde (liquid) dip used in 1930 and 1931 proved the most effective. It completely eliminated smut in every seed lot.
 - B. The Dubay dusts No. 655 and No. 665 used in 1930 and 1931 reduced smut to zero in 1930 and to 0.01 per cent in 1931. In the 2-year test the plants from seed treated with each of the dusts produced approximately 28,000 heads, all smut-free except one. The plants from nontreated seed in 1930 and 1931 produced 2,876 smutty heads in approximately 28,000.
 - C. The formaldehyde dusts, Smuttox, Kantsmut, and Corona Oat Dust, tested in 1930 and 1931, were next in effectiveness, reducing smut to 1 per cent or less in every seed lot and to zero in most of the lots.
 - D. Ceresan, tested in 1930 and 1931, produced excellent control except that in 1 lot in the first sowing at Aberdeen, Idaho, in 1930, plants from Ceresan-treated seed produced 18 smut heads in 650 (2.8 per cent). Plants from nontreated seed of this lot produced 24.2 per cent smutty heads. With this exception, Ceresan eliminated smut or reduced it to a trace.
 - E. K-1-C, tested in 1928, produced fairly satisfactory control. K-1-D, also tested in 1928, was less effective. These dusts, the experi-

mental forerunners of Ceresan, are similar to the latter but contain less of the toxic ingredient (ethyl mercury chloride).

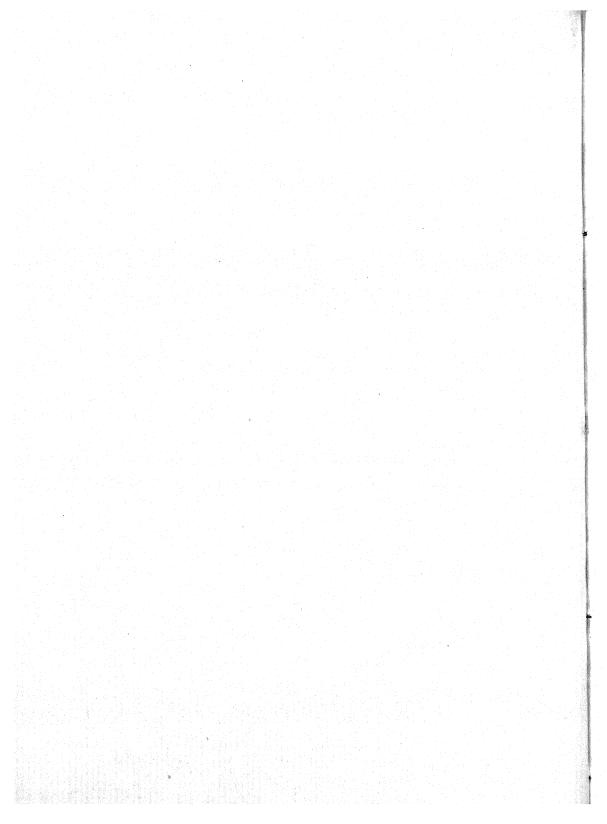
F. Ansbacher Grain Dust and Wa Wa Dust, tested in 1930, were effective in the sowing at Arlington Experiment Farm and in the second sowing at Aberdeen, Idaho, but these dusts produced unsatisfactory control in the first sowing at Aberdeen.

- G. The following dusts proved unsatisfactory: Abavit B, Acco, Anchor Sulfur, D.D.D. No. 68, Niagara sulfurs Nos. 76, 77, 78, and 79, Sterocide, Tillantin Höchst, and Tutan. Plants from seed treated with Acco dust, in 1930, produced more smut than those from nontreated seed in 5 of the 10 lots grown at Arlington Experiment Farm and in all of the 10 lots in both sowings at Aberdeen, Idaho.
- 3. With regard to the effects of the various treatments on seedling emergence, the formaldehyde (liquid) dip reduced emergence from 93.3 per cent to 88.8 per cent in 1931. With this exception none of the effective treatments produced appreciable reduction.
- 4. Facts showing the importance of oat-smut control are: (1) the appearance in recent years of new physiologic forms of the loose- and covered-smut fungi that attack important varieties hitherto highly resistant or immune and (2) the estimated annual loss from oat smut approximating 50,000,000 bushels.

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RESISTANCE OF MONOCOTYLEDONS TO PHYMATOTRICHUM ROOT ROT $^{\scriptscriptstyle 1}$

J. J. TAUBENHAUS AND WALTER N. EZEKIEL

Phymatotrichum root rot attacks a considerable proportion of the cultivated dicotyledonous plants (6), but it has been generally assumed, even by growers, that monocotyledonous plants, such as corn, sorghum, Sudan grass, and grass and grain crops in general, are resistant to the disease. In the first scientific description of the disease by Pammel (4), it was noted that: "So far as is known the fibrous-rooted plants, such as corn, oats and wheat, are exempt from the disease, and where practicable these should follow cotton." In field studies of graminaceous crops, over a period of 14 years, in all parts of Texas, the writers have not found a single monocotyledonous plant that showed lesions of Phymatotrichum root rot or that had succumbed to the disease. Graminaceous crops have therefore been recommended (11, 10) as nonsusceptible crops to be used in rotations where root rot occurs.

Recently, however, King and Loomis (3) have emphasized that the Phymatotrichum fungus will grow in artificial cultures on graminaceous roots, and they report also having found Phymatotrichum strands occurring naturally on the roots of date palms, Johnson grass, and Bermuda grass. They considered this as evidence that monocotyledonous plants are hosts to root rot and suggested that plants of this sort may act as carriers of the disease. It has therefore appeared necessary to study further the question of whether monocotyledonous plants may be hosts to this disease. This is of particular importance, since, unless monocotyledonous plants are resistant to Phymatotrichum root rot, there are practically no crops that can be recommended for rotations for the control of the disease.

PRELIMINARY INOCULATIONS OF SORGHUM PLANTS

In the summer of 1928, sorghum and cotton plants were grown in separate containers filled with Lufkin fine sandy-loam-soil material to which was added 1 per cent of ground limestone to make the soil favorable for root rot. On September 8 and again on October 9 the plants in 4, each, of the cotton and sorghum containers were copiously inoculated with Phymatotrichum root rot. The method of inoculation as described in more detail elsewhere (5) consisted of placing freshly infected cotton roots in holes next to the plants to be inoculated. Two-containers, each, of cotton and sorghum plants were left noninoculated as checks. Within 20 days, the inoculated

¹ Published with the approval of the Director as Contribution No. 174, Technical Series, of the Texas Agricultural Experiment Station.

cotton plants had succumbed to typical Phymatotrichum root rot, while the noninoculated cotton plants remained normal throughout the season. Neither the inoculated nor the noninoculated sorghum plants showed any evidence of infection but remained normal and continued to grow. On November 8, 1928, the roots of all the inoculated and noninoculated cotton and sorghum plants were washed out from the soil and carefully examined for root decay and for the presence of Phymatotrichum strands. Roots of the inoculated cotton plants were found to be decayed, with typical symptoms of root rot and numerous Phymatotrichum strands that clung to the infected roots and had not washed off in washing the soil from the roots. On the other hand, the roots of the noninoculated cotton plants, as well as the roots of both the inoculated and noninoculated sorghum plants, showed no evidence whatever of root decay or Phymatotrichum strands.

On the roots of both the check and the inoculated sorghum plants there were distinct, dark lesions, penetrating deeply into the roots, which might have been considered as possibly caused by Phymatotrichum root rot. Very exhaustive microscopic study of these sorghum root lesions failed to reveal any evidence of Phymatotrichum growth. A number of Petri-dish cultures were made from these lesions as well as from lesions on cotton roots that had been inoculated with Phymatotrichum root rot. Typical Phymatotrichum growth was readily recovered from the cotton-root lesions, while the sorghum root lesions invariably yielded a species of Fusarium and a Rhizoctonia, with no Phymatotrichum from either inoculated or noninoculated sorghum plants. These preliminary results suggested strongly that sorghum, a monocotyledonous plant, is not susceptible to root rot.

EXPERIMENTS IN 1930

More recent experiments have included inoculations of a number of plants growing in field plots (8, 9); inoculations of potted plants in the laboratory; and also inoculations of fleshy storage bodies in moist chambers in the laboratory.

Inoculations in field plots. It appeared desirable to repeat the preliminary work reported above more extensively, using a larger number of monocotyledonous plants. Early in the spring of 1930 some field plots were prepared in the senior writer's home garden at College Station. The soil in this garden is a typical Lufkin fine sandy loam with a pH of 7.0–7.6 and thus well within the range found favorable for root rot (1). However, to insure conditions highly favorable for root rot, finely ground limestone at the rate of 1 ton per acre was incorporated into the soil. It is to be noted that susceptible crops had been grown in this garden previously for at least 10 years without any evidence of root rot; artificial inoculations were therefore the sole source of infection in these tests, without any variability from

such local differences in prevalence of the disease as may be expected when natural infection is depended on.

The land available was divided into 5 plots, each about 12 x 36 ft., and planted to the monocotyledons. These presumably resistant plants were interplanted with carrots, cotton, or okra, which are highly susceptible to root rot. The monocotyledonous plants included corn, Zea mays; cannas, Canna indica; Caladium, Colocasia illustris; Iris, Iris sp.; tuberose, Polianthes tuberosa; gladiolus, Gladiolus sp.; tiger-lily, Lilium tigrinum; pink zephyr lily, Zephyranthes (Atamosco) rosea; Tritonia, Tritonia (Montbretia) sp.; day lily, Hemerocallis sp.; nut grass, Cyperus rotundus L.; hedge bamboo Bambusa nana; evergreen shallot, Allium ascalonicum; and 3-year-old date palms, Phoenix dactylifera. Parallel plantings of corn and cotton were made at intervals throughout the season (Table 2). When the plants were fully grown, the monocotyledons as well as the susceptible dicotyledons were inoculated periodically with fresh inoculum of naturally infected carrot or cotton roots. Detailed results are given in tables 1 and 2, except for the date palms, which showed no aboveground symptoms of

TABLE 1.— Results of 1930 field-plot inoculations with Phymatotrichum root rot, including miscellaneous monocotyledonous plants with carrot and okra plants as checks [Planted Feb. 18-March 3, 1930, except nut grass, which was present as a weed]

			Results	1
Plants	Dates of inoculation	Date of observa- tion	Total number of plants	Percentage of plants in- fected with Phymatotri- chum root rot
Gladiolus	April 26, May 12, July 7	Aug. 2	460	()a
Hedge bamboo	April 26, May 12, July 7	Nov. 29	12	0
	Aug. 10, Sept. 11			
Iris	[1] [[2] 함께 바다 하게 함께 하는데	"	12	0
Shallot		"	150	0
Carrot	May 12, July 7	Aug. 2	100	97
Okra	May 12, June 15, July 7	"	100	100
Caladium	May 12, July 7, Aug 10	Aug. 27	50	0ь
Canna	May 12, July 7, Aug. 10,	Oct. 7	50	0
	Sept. 11			
Day lily	[[하는 기가 하얗 시간 12]	Nov. 10	50	0
Mexican tuberose	[요즘 얼마는 사람들이 네스트 글리스트	"	50	0
Tritonia	[조명이 회사장님 (450 회문) - 교육요.	"	50	0
Tiger lily	[이름 : 기타를 보면 다양하는 생각으로	6.6	50	0
Zephyr lily, pink			50	0
Nut grass	Aug. 2, Sept. 5, Sept. 20	Nov. 29	100	0,

² Some of mother bulbs and lateral roots infected with Pythium dry ro

b Some of Caladium roots showed lesions caused by Sclerotium rolfsii.

TABLE 2.—Field-plot inoculations with Phymatotrichum root rot in 1930 of cotton and corn planted periodically

				Results	
Dates of planting	Plant	Date of inoculation	Date of observa- tion	Total number of plants	Percentage of plants with Phymatotrichum root rot
May 5	Corn Cotton	June 20, July 7, Aug. 2	Nov. 11	240 240	0 100
June 5	Corn Cotton	Aug. 2, Sept. 5, Sept. 20	Nov. 29	200 200	0 100
August 5	Corn Cotton	Sept. 5, Sept. 20	Oct. 9	240 240	0 96
Sept. 8	Corn Cotton Cotton	Oct. 11, Oct. 28	Nov. 29	140 140 100	0 79 0

root rot and were left in the ground for further testing. None of the roots of the monocotyledonous plants tested showed the least evidence of Phymatotrichum root rot. On the other hand, high percentages of the inoculated carrot, okra, and cotton plants succumbed to typical Phymatotrichum root rot (Fig. 1, A, B, and D). These susceptible, dicotyledonous plants had been interplanted between the monocotyledons throughout all 5 plots, and the uniform infection of these dicotyledonous plants proved that conditions throughout the entire area had been favorable for the development of the disease. The absence of root rot on the monocotyledons was thus strong evidence of the resistance of these plants to the disease.

Lesions on graminaceous roots. When the roots of the dicotyledonous and monocotyledonous plants were dug out at the end of the season typical Phymatotrichum root-rot lesions were found on the roots of the dicotyledonous plants, but there were also dark lesions on the roots and rootlets of inoculated and also of noninoculated corn plants. These lesions appeared similar to those found previously on sorghum roots. Many corn roots showing such lesions, as well as the roots of dicotyledonous plants infected with typical Phymatotrichum root rot, were cultured by the soil-culture method. This method, described elsewhere (7), consists of placing roots in jars filled with moist soil. The writers used quart Mason jars of Houston clay soil with 25 per cent moisture on a dry-weight basis. With roots affected by Phymatotrichum root rot, the strands of the fungus grown out



Fig. 1. Inoculation experiments with Phymatotrichum root rot. A and D. Cotton and corn plants, interplanted in same row in A and in alternate rows in D. All inoculated. Cotton plants dead or dying from root rot and corn plants normal. B. Canna and okra planted alternately in the same row and inoculated. Okra plants dead from root rot and canna plants normal. C. Lesions on corn root, as found in root-rot and root-rot-free areas and proved distinct from Phymatotrichum root rot.

and are readily visible along the sides of the glass. Such strands frequently also produce an abundance of Phymatotrichum sclerotia. This method has made it possible to determine whether root lesions were the result of Phymatotrichum infection, thus supplementing standard methods of isolation and pure culture.

The results of these cultures are summarized in table 3. No Phymatotrichum growth appeared in the soil cultures from either the inoculated or the noninoculated corn roots. However, copious Phymatotrichum strand growth and sclerotia were secured from the inoculated cotton roots. In cultures in which Phymatotrichum-infected cotton roots were mixed with the corn roots, Phymatotrichum growth was obtained only from the infected cotton roots and not from the corn roots. This was proved by breaking open the glass containers and carefully dissecting the soil to determine from which of the roots the Phymatotrichum strands were coming.

In addition to the soil cultures mentioned, series of test-tube cultures also were made from the Phymatotrichum-infected cotton roots and from many of the lesions of the corn roots growing in the same plots. Pieces of root tissue sterilized ½ minute in 1–2000 mercuric chloride in 25 per cent alcohol and rinsed in sterilized water were inserted in slants of agar. Typical Phymatotrichum root-rot growth was always recovered from the Phymatotrichum-infected cotton roots but never from the check, noninoculated cotton roots, nor the lesions on the corn roots. Instead, colonies of Fusarium, Rhizoctonia, and several other undetermined organisms grew from the corn-root lesions. From this it seems evident that the lesions found on the roots of inoculated corn plants were not caused by Phymatotrichum root rot.

Extensive field studies were made in 1928, 1929, 1930, and 1931 of corn. sorghum, and other graminaceous plants found growing in different sections of Texas, not only in root-rot-infected areas but also in parts of east Texas and on individual farms in Brazos County where root rot has not been known to occur. Whether secured from root-rot or root-rot-free areas. the roots of the corn, sorghum, nut grass, and Johnson grass frequently showed numerous dark reddish lesions resembling those found on the corn roots from the plots (Fig. 1, C). Many of these lesions were cultured, using the soil-chamber method. As noted in table 3, no Phymatotrichum growth ever appeared from any of the lesions on roots of corn, sorghum, Johnson grass, or nut-grass plants. On the other hand, copious Phymatotrichum strand growth and heavy sclerotia formation were always obtained from cotton roots infected with typical root rot, whether such roots were artificially or naturally infected. These various series agreed in indicating that lesions found on the roots of various graminaceous plants are not caused by Phymatotrichum root rot.

TABLE 3.—Results of soil-chamber cultures of roots of monocotyledons and dicotyledons exposed to artificial or natural infection with Phymatotrichum root rot [Incubated at 20-34° C., mean = 27.8° C.; final notes taken Jan. 14-29, 1931]

Material cultured	Date of culturing	Total num- ber of	Results: of jars v matoti	vith Phy- ichum
		jars	Strands	Sclerotia
From plot experiments:	1930			
Corn roots	Sept. 11 Oct. 9 Dec. 2	83 24 8	0 0 0	0 0 0
Cotton roots	Sept. 11 12	14 9	14 9	10 9
	Oct. 9 Dec. 2	12 5 12	$\begin{array}{c c} 12 \\ 5 \\ 12 \end{array}$	10 4 10
Cotton and corn roots together in jars	Sept. 12 Oct. 9	9 12	9a 12a	7 10
Evergreen shallot	Dec. 9	17 16 16	0 0	0 0
From W. Conrad farm, Brazos County, where Phymatotrichum root rot absent: June corn roots with lesions Sorghum roots with lesions Nut-grass roots with lesions Cotton roots with Fusarium wilt	Dec. 15	48 24 12 12	0 0 0 0	0 0 0 0
From Williams farm, Brazos County, where Phymatotrichum root rot abundant:				
June corn roots with lesions	Dec. 16	48 48	0	0
Johnson grass roots with lesions Cotton roots with Phymatotrichum root rot Cotton roots with root rot together in jars	"	24 12	$\begin{array}{c c} & 0 \\ 12 \end{array}$	0 11
with sorghum roots with lesions	"	12	12a	12
From Bryan, Texas, root-rot-free area: June corn roots with lesions Lyne corn roots together in jorg with eat	Dec. 17	48	0	0
June corn roots together in jars with cotton roots from root-rot spots		15	15a	15

^a Phymatotrichum strands growing out from cotton roots only.

Soil cultures were made also from the inoculated Caladium, evergreen shallots, cannas, and lilies that were grown in the plot experiments. No Phymatotrichum growth appeared from any of these plants.

Inoculations in the laboratory. In order to test further the resistance of monocotyledons to Phymatotrichum root rot, 2 series of inoculations were carried out in the laboratory. On December 2, 1930, a number of carrots and onions were carefully washed in tap water, dipped in 1-1000 bichloride

of mercury solution, rinsed with sterilized water, and then placed in separate moist chambers. With a flamed scalpel 2 slits were made in each carrot root and onion bulb, and sclerotia from a pure culture were inserted in these slits. Definite Phymatotrichum decay was obtained with 13 of the 16 inoculated carrots, and doubtful infection in 2 others; while not one of the 20 inoculated onion bulbs showed any decay. The check, noninoculated carrots (16 roots) and onions (20 bulbs) also remained normal.

Further inoculations were carried out with Irish potatoes, carrots, onions, hyacinths, cannas, and tuberoses planted in 5-inch pots filled with sifted Houston black clay-soil material. All these plants were inoculated with sclerotia from pure cultures, through scalpel punctures, as in the moist-chamber series above. After 6 weeks, the plants were removed from the soil and examined carefully for any evidence of Phymatotrichum infection. It is to be noted (Table 4) that the Irish potato and the carrot, both

TABLE 4.—Puncture inoculations, with sclerotia of Phymatotrichum omnivorum, of monocotyledonous and dicotyledonous plants growing in small pots
[Inoculated Feb. 26, final results April 7, 1931]

		Inoculated	Checks, r	ioninocu- ced
Plant	Number of pots	Results	Number of pots	Results
Irish potato	2	Typical root-rot decay	2	Normal
Carrot	3		2	"
Onion	3	Normal	3	
Hyacinth, Paper White	4		4	
Canna	4	•	4	"
Tuberose	4		4	"

dicotyledonous plants, developed typical Phymatotrichum decay; while the onion, hyacinth, canna, and tuberose, all monocotyledonous plants, remained normal. Thus, even puncture inoculations of these monocotyledonous plants, in soil as well as in moist chambers, have failed to demonstrate any susceptibility to Phymatotrichum root rot under conditions that allowed typical decay of the susceptible, dicotyledonous plants.

DISCUSSION

The resistance of monocotyledonous plants to Phymatotrichum root rot is of great importance in the planning of field control methods. The data presented in the present paper summarize inoculations of 16 kinds of mono-

cotyledonous plants, carried out under both field and laboratory conditions. No infection was obtained with any of these plants, while high percentages of infection were secured with cotton, okra, and carrot plants growing side by side with the monocotyledonous plants.

On 1 or 2 occasions we have found Phymatotrichum strands on roots that may have been Johnson-grass roots, found growing in cotton or alfalfa fields and intimately intermingled with root-rot-infected cotton or alfalfa But, we do not deem this sufficient evidence to consider Johnson grass a host of root rot. It seems more probable that graminaceous roots growing intermingled with the roots of infected susceptible plants may occasionally serve as the physical substratum for a stray strand of the fungus without this being of any pathologic significance. According to the usual conception, susceptibility to a fungus assumes the possibility of definite infection of the plant by the fungus. For a plant, even though resistant to infection, to be a "carrier," should be able to further in some way the survival of the fungus. This term could be applied to a plant that aids in the growth of the fungus or harbors it thereafter over a more extended period of survival than would be possible otherwise but could scarcely be applied to a plant that serves only rarely, if at all, as the completely inert substratum for stray fungus strands. As has been shown above, none of the monocotyledonous plants inoculated have become infected, nor have their roots served as carriers to transfer the fungus to soil cultures or to cultures on ordinary media. The monocotyledonous plants studied have thus far furnished no evidence to justify any change in the assumption that monocotyledonous plants are neither hosts nor carriers of Phymatotrichum root rot. It may be noted that preliminary studies on the physiologic nature of the resistance of monocotyledonous plants to root rot, reported in an accompanying paper (2), indicate that there is some actual toxic substance in the juices of monocotyledonous plants that inhibits the growth of Phymatotrichum omnivorum.

Typical dark lesions were found on roots of inoculated and of check corn and sorghum plants in these experiments and also on roots from plants secured from root-rot and root-rot-free areas in fields. Isolations made from such lesions invariably yielded a Fusarium, a species of Rhizoctonia, and other organisms not yet identified, some of which may be the cause of the trouble. The roots of many of the graminaceous crops in Texas, while not attacked by Phymatotrichum root rot, are thus affected by other root diseases that will be the subject of further study.

SUMMARY

A large number of monocotyledonous plants were grown in field plots side by side with susceptible okra, carrot, and cotton plants and copiously inoculated with Phymatotrichum root rot. In the laboratory, carrot roots

and onion bulbs were inoculated in moist chambers. Carrots, onions, hyacinths, cannas, and tuberoses were grown in 5-inch pots and inoculated with sclerotia of Phymatotrichum omnivorum taken from pure cultures. In all cases the carrot, okra, and cotton plants succumbed to typical root rot and the causal organism was readily recovered. On the other hand, not a single plant of the 16 different genera of monocotyledonous plants inoculated became infected, succumbed to the disease, or even showed traces of the Phymatotrichum strands on the roots.

Definite, dark reddish brown lesions were found penetrating deeply into the noninoculated as well as the inoculated corn and sorghum roots. Similar lesions were found also on roots of the same plants, as well as on roots of Johnson grass and many other graminaceous plants, growing naturally in root-rot or root-rot free areas. Petri-dish, test-tube, and soil cultures made from these lesions indicate definitely that they were not caused by Phymatotrichum root rot but were induced by other microorganisms.

From the results presented it appears that those monocotyledonous plants tested are neither hosts nor carriers of Phymatotrichum root rot.

COLLEGE STATION.

TEXAS.

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RESISTANCE OF THE TURK'S-CAP HIBISCUS, MALVAVISCUS CONZATTII, TO PHYMATOTRICHUM ROOT ROT¹

WALTER J. BACH AND J. J. TAUBENHAUS

Root rot that is caused by the fungus, *Phymatotrichum omnivorum* (Shear) Duggar, is known to attack over 550 species of cultivated and non-cultivated plants.² In the study of host relationship to Phymatotrichum root rot, it is necessary to determine the susceptibility of cultivated as well as noncultivated plants. It is also imperative to find, if possible, resistant strains or varieties of plants that may be used in areas where the disease is prevalent. The present paper presents data on the relative susceptibility of various plants of the family Malvaceae to Phymatotrichum root rot under field conditions, as well as inoculation experiments with the Turk's-cap hibiseus.

Intensive field studies and observations have been made over a period of years on the degree of resistance or susceptibility of malvaceous plants found growing in Texas to Phymatotrichum root rot.³ Such plants included cultivated as well as noncultivated species and varieties. In general, plants belonging to the Malvaceae, such as cotton, okra, and roselle, were found to be very susceptible. The only resistant species found in this family was the Turk's-cap hibiscus, *Malvaviscus conzattii* Greenman.

In determining the susceptibility of malvaceous plants growing under field conditions favorable to Phymatotrichum root rot, the susceptibility of the plants studied was ascertained by actual wilting of the plants and by a careful examination of their root systems. Wilting alone is not always a reliable symptom of root rot, since this might be brought about by insect injury, drouth, heat, or other cause. The presence of Phymatotrichum root rot was determined by the wilting of the affected plants and by the presence of the yellowish to buff strands of the fungus mycelium on the infected roots. With woody plants or shrubs, it is sometimes difficult to establish the presence of Phymatotrichum root rot. In such cases, infected plants, even though they do not wilt, shed their leaves rather quickly and not infre-

¹ The writers are indebted to the late Professor H. Ness, Chief of the Division of Botany, Texas Agricultural Experiment Station, and Professor V. L. Cory, Grazing Research Botanist, Substation No. 14, for verification and identification of many of the plant species included in table 1.

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² Taubenhaus, J. J., B. F. Dana, and S. E. Wolff. Plants susceptible or resistant to cotton root rot and their relation to control. Texas Agr. Exp. Sta. Bul. 393. 1929.

³ Bach, W. J., and J. J. Taubenhaus. The resistance of *Malvaviscus conzattii* (arboreus) to Phymatotrichum root-rot. (Abst.) Phytopath. 21: 120. 1931.

quently recover by producing new growth. Hence, in dealing with shrubs or woody plants, the roots were given detailed microscopic examination.

Rooted cuttings and more mature plants of the Turk's-cap hibiscus were grown in nursery rows for the inoculation experiments. These plants were inoculated repeatedly. The method of inoculation consisted of inserting roots of freshly wilted cotton or carrot plants in contact with the roots of the plants to be inoculated.

FIELD OBSERVATIONS ON THE RELATIVE SUSCEPTIBILITY OF PLANTS OF THE MALVACEAE FAMILY TO PHYMATOTRICHUM ROOT ROT

Table 1 lists the species of the Malvaceae that have been studied from the standpoint of susceptibility to Phymatotrichum root rot. This list is an alphabetical arrangement of both the cultivated and noncultivated plants. It will be seen from table 1 that, with the exception of the Turk's-cap hibiscus, all the plants of the Malvaceae observed were found susceptible to the disease, some of the plants were highly susceptible, while others were only slightly to moderately so.

RESISTANCE OF TURK'S-CAP HIBISCUS TO PHYMATOTRICHUM ROOT ROT

It has been shown in table 1 that the Turk's-cap hibiscus, under field conditions, is apparently resistant to Phymatotrichum root rot. Although this plant is a perennial woody shrub grown extensively in the Lower Rio Grande Valley as an ornamental or hedge plant (Fig. 1, a) it is rather sensitive to cold and is therefore adapted only to southern Texas and lands of similar climate. The plant is readily propagated by cuttings. Table 2 shows the results of inoculations on Turk's-cap hibiscus. A total of 1.066 attempts were made to inoculate individual Turk's-cap plants, ranging in age from rooted cuttings to full-grown plants. Of this number only 7 of the recently transplanted cuttings showed infection and 6 of these recovered entirely. Two out of 6 1-year-old plants inoculated at College Station showed infection but recovered later. None of the well-established plants succumbed to the disease or showed signs of infection on examination of their root systems. During the 1930 season the Turk's-cap plants were interplanted with cotton, and both the cotton and Turk's-cap were inoculated. The cotton was killed, while the Turk's-cap plants did not succumb (Fig. 1, b). An examination of the root system later failed to show any sign of infection of the latter host.

On December 28, 1929, the entire lot of 142 Turk's-cap plants, which had been inoculated at various times from June 12, 1928, to September 30, 1929,

⁴ Taubenhaus, J. J., B. F. Dana, W. N. Ezekiel, W. J. Bach, and J. P. Lusk. A method of inoculation for Phymatotrichum root rot investigations. Phytopath. 19: 167–170. 1929.

TABLE 1.—Field observations on the relative susceptibility of various cultivated and non-cultivated malvaceous plants to Phymatotrichum root rot

Name of plant	Relative suscep- tibility ^a	1	Name of plant	Relative suspec- tibility
Abutilon abutilon (L.) Rusby	+	Malva parvifi	ora L.	++
'' berlandieri A. Gray	+		lifolia L.	+
" incanum (Link.) Sweet	+	" sylvest	tris L.	++
" malacum S. Wats	+		americanum (L.) Torr	+
" megapotamicum St. Hil. Naud.	+		coccinum (Pursh.) A.	
" parvulum A. Gray	+	"	eptophyllum A. Gray	+ +
ii pictum Walp.	+		spicatum (L.) A. Gray	1 +
texense T. G.	+	Malvavisana	arboreus Cav	++
" triquetrum (L.) Presl.	+		onzattii Greenman	
" wrightii A. Gray	1		drummondii T. G.	+
Althaea ficifolia Cav.	++		liniana (L.) G. Don.	+
'' rosea Cav.	++		petala Schwele.	++
Anoda lavaterioides Medic.	++		folia Lam.	+
Bastardia viscosa (L.) H.B.K.	+		L.	++
Callirrhoe digitata Nutt.	++		ia A. Gray	++
" geranioides Small	+++		H.B.K.	++
" involucrata (Nutt.) A.			is Morie.	++
Gray	++		A. Gray	++
" lineariloba (T. & G.) A.			St. Hil.	+
Gray	+		ea Torr.	+
" papaver (Cass.) A. Gray	++		Rose	+
" pedata A. Gray	+	" longipes	A. Gray	+
" scabriuscula Robinson	++		cana A. Gray	+
" triangulata (Leavenw.)		" physoca	lyx A. Gray	++
A. Gray	+	" rhombif	olia L.	+
Cienfuegosia sulphurea (St. Hil.)			L	++
Garcke	++		(T. & G.) Small	+
Disella hederacea (Dougl.) Greene	+		lia L	++
Gayoides crispum (L.) Small	+		ista (A. Gray) Wott.	
Gossypium barbadense L.	++		nd	++
' herbaceum Small	++	Sphaeralcea	cuspidata (A. Gray)	
misutum L	++	6.6	Britton	+
peruvianum Cav	++		fendleri A. Gray	+
Hibiseus eardiophyllus A. Gray	+		hastulata A. Gray	++
coccineus wait.	++	"	incana Torr.	+
esculentus L	++		lindheimeri A. Gray	++
maiiii0 11	+		lobata Wott.	++
rosa-sinensis Li	++		pedata Torr.	+
sabuarina II	++		pedatifida A. Gray	+
syriacus L	++		pumila Wott. & Stand.	+
trionum L	++		subhastata Coult.	+
Kostelezyka althaeifolia (Chapm.)			tenuipes Wott. & Stand.	+
A. Gray	+	Wissadula	holosericea (Schwele.)	
Malachra capitata L.	+	"	lozani (Rose) Fries	, +
		"	periplocifolia(L.)Griseb.	++
			herrhogma(n.) ausen.	+

Highly susceptible.

Moderately to slightly susceptible.

Resistant.

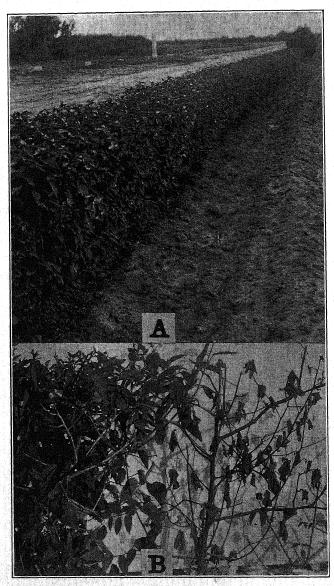


Fig. 1. Resistance of Turk's-cap hibiscus to Phymatotrichum root rot. A. Turk's-cap hedge that has grown vigorously for 3 years without showing any evidences of root rot, although the disease has been prevalent on both sides for several years. B. Uninjured Turk's-cap plant (to left) and dead cotton plant (to right), representative of a row of interplanted plants copiously inoculated with Phymatotrichum root rot.

TABLE 2.—Resistance of Turk's-cap hibiscus to infection by Phymatotrichum root rot

ν.	Condition of roots of inoculated plants on examination	punos	,,,	•		,,	,	•				:		•	,,,		,,	Decayed		•		Sound	,,,	
Results of inoculations	Number of plants recovered																H	0		0		4	2	
Results of	Number of days from in- oculation to infec- tion																12-19	4-96		26		26	56	
	Number of plants infected	0	0	0	0	0	0	0	0	0	0	0		0		>	က	2.2		22		4	c 1	
	Date of inoculations	6-12-28	6-12-28	7-30-28	8-30-58	10-12-28	10-30-28	6-12-59	7-12-29	8-8-29	8-20-59	9-30-59	3-8-30	4- 1-30	7- 7-30	7-24-30	7-24-30	7-24-30	9- 1-30	10-14-30	9- 1-30	10-14-30	9- 1-30	10-14-30
	Number of plants inoculated	12	12	20	50	142	53	142	142	142	142	142a	37		37b	909	rea.	77	66		12b	ŀ	6 p	
	Source of inoculum	Cotton	Carrots	Cotton	3	,,,	,,,	•	,,,	,	,,,		Carrot		Cotton	,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,		j	3			•		
	Age of plant inocu- lated	1 yr.	,	,,,	,,	,,	99	14 yrs.	2 yrs.	,,	;	•	Rooted	cuttings) 3					Doctod	nannour	cuttings	1 yr.	
	Host inocu- lated	Turk's-	cap ,,		,,,	,,	,,,	2,7	•	,,,	23	3			•	,		Cotton		m	-s wint	cap		
	Place of inocula- tion								Weslaco,	Texas										F	Correge	Station,	Texas	

a All above dug out and roots examined 12-28-29.

^a Interplanted with cotton and both Turk's-cap and cotton inoculated.

as shown in table 2, were dug out and the root systems examined for evidence of the Phymatotrichum root-rot fungus. The roots of these plants showed no signs of infection. However, it was found that when rooted cuttings of Turk's-cap were planted in the field and immediately inoculated, such plants might become infected. During November, 1930, all of the inoculated Turk's-cap plants at College Station were dug out and examined. Only 4 of the rooted cuttings showed infection. However, by the time these plants were dug out, they had made a new root system and the old infected roots had almost disappeared. Other plants showed very slight signs of infection on the roots; but, apparently, the plant itself was unaffected, for the foliage remained green and luxuriant and no wilting of any kind was noticeable.

From these inoculation experiments, it is concluded that the Turk's-cap hibiscus is highly resistant to Phymatotrichum root rot. It is still unknown whether this apparent resistance is of a physiologic nature or whether it is due to morphologic differences between the roots of this plant and other malvaceous hosts that are highly susceptible to Phymatotrichum root rot. Further research is now in progress and will be reported later.

SUMMARY

In general, plants belonging to the Malvaceae have been found highly susceptible to Phymatotrichum root rot. The Turk's-cap hibiscus appears to be an exception, as it was found to possess a very high degree of resistance. Eighty-two species of plants belonging to the family Malvaceae have been observed and their relative susceptibility to Phymatotrichum root rot indicated. The Turk's-cap hibiscus is the only one of these plants observed to show no infection under field conditions. One thousand and sixty-six attempts at artificial infection of this plant failed to cause infection and death of well-established plants. Further studies are in progress to determine the factors and nature of this resistance.

COLLEGE STATION, TEXAS.

GROWTH OF PHYMATOTRICHUM OMNIVORUM IN PLANT JUICES AS CORRELATED WITH RESISTANCE OF PLANTS TO ROOT ROT¹

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Phymatotrichum omnivorum (Shear) Duggar is an aggressively parasitic fungus, which causes a destructive root rot within a wide range of host plants. Plants susceptible to the disease include such diverse ones as cotton, sweet potato, alfalfa, grape, persimmon, fig, morning-glory, aster, and more than 500 other plants (11). Yet, so far as is known, all of these susceptible plants belong to either the Gymnospermae or Dicotyledoneae. No monocotyledonous plant has yet been found definitely susceptible to Phymatotrichum root rot, as shown in detail elsewhere (12, 13). There would thus appear to be some general difference between dicotyledonous and monocotyledonous plants that enables the root-rot fungus to parasitize perhaps the majority of dicotyledonous plants, as well as many gymnosperms, but makes it difficult or impossible for P. omnivorum to attack successfully any of the monocotyledonous plants. Such a difference might be due to morphologic peculiarities preventing infection or growth of the fungus in monocotyledons, or to biochemic peculiarities encouraging growth of the fungus in the case of the susceptible plants or inhibiting it in the case of the resistant plants.

Some preliminary tests of the comparative nutritive value to the fungus of juices expressed from the roots of root-rot-resistant corn plants and of susceptible cotton plants showed much better growth in the juice from the susceptible cotton roots (3, 4). Further experiments have since been carried out with juices from other plants. The work has so far included 4 monocotyledonous plants, all resistant to root rot, and 4 dicotyledonous plants, all susceptible to root rot. Other studies on the same general problem will consider the possible morphologic factors that may be involved in susceptibility or resistance to root rot, and will be reported separately.

HISTORICAL

There appears to be no record in the literature of previous work on the nature of resistance to Phymatotrichum root rot. However, there has been much work on the nature of resistance to other plant diseases (14). Only a few recent studies concerned with the type of work reported in the present paper will be noted here.

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The most outstanding contributions have been those of Walker and his associates (15, 1) on the basis of resistance to onion smudge. The resistance of red and yellow onions to this disease was proved due to toxic material present in the dry outer scales of these resistant bulbs and apparently consisting, at least in part, of protocatechuic acid. So far as the writers know, this is the only recorded study of physiologic resistance to a disease that has progressed to the discovery of the precise chemical nature of the material apparently involved in resistance.

A number of investigators, however, have found that resistance to the plant diseases with which they worked could apparently be explained by materials extractable from the normal plant tissues, even though these materials have not vet been isolated or identified. Thus, Reynolds (9, 10) found that growth of Fusarium lini was depressed in an extract from wiltresistant flax more than by an extract from a susceptible variety. Working with bark diseases of sour-orange and lemon trees, Klotz (5) found that resistance to the diseases correlated with an inhibitory effect of the bark on certain enzymes produced by the fungi and suggested that similar inhibition of fungus enzymes by plant tissues might explain disease resistance in other plants. Ranker (8) observed inhibition of growth of Ustilago zeae in the expressed juices of some but not all of a series of selfed lines of corn resistant to smut, while growth of the fungus was not interfered with by juices from any of the susceptible lines of corn tested. Newton and his associates (6, 7) noted that stem-rust infection of detached leaves of susceptible wheat varieties was in some cases reduced by contact. in the Petri-dish cultures, with extracts from resistant varieties. Ezekiel (2) found that extracts from wheat varieties differed in their ability to support the growth of physiologic forms of Puccinia graminis tritici in agreement with the respective resistance or susceptibility of the varieties to the various forms, although a group of the physiologic forms yielded aberrant results so consistently as to suggest that there are at least 2 series of relations involved in resistance to stem rust.

METHODS AND MATERIALS

The general plan of the work reported herewith has been to compare the growth of *Phymatotrichum omnivorum* on juices prepared by the same methods and at the same time from plants susceptible to root rot and from plants resistant to the disease. Large series of cultures prepared from the plant juices were inoculated at the same time and incubated side by side, and colonies harvested from the various series after the intervals specified. The dry weight of the fungus mat was determined, individually for each culture in which there was perceptible growth, by drying the fungus materials in crucibles in the oven.

Except where otherwise specified, the roots used in the preparation of the plant juices were from plants grown at College Station, Texas, and harvested immediately before being used. In all cases, only the underground portions of the roots or storage bodies were used, any surface or greened portions as well as injured portions being discarded. Roots were washed in tap water, rinsed in distilled water, trimmed, and ground in a fine grinder, and the juice then expressed with a hand press. The liquid obtained was strained through absorbent cotton by suction. Portions to be ultrafiltered were removed at this time and passed through a Berkefeld filter, and the ultrafiltered juices were handled thereafter with sterile pipettes and appropriate aseptic precautions. Inasmuch as the juices could be ultrafiltered only with great difficulty, however, most of the series of cultures were run with juices sterilized by heat instead of ultrafiltration. For such series, the undiluted juice was autoclaved for 25 min. at 10-lbs. pressure; refiltered through cotton to remove the coagulum, often precipitated by this first autoclaving; and the juice was then distributed to the individual culture flasks, which were plugged as usual with cotton and autoclaved for 20 min. at 10-lbs. pressure.

Synthetic nutrient solution 70, used in much of this work as a diluent for the juices, is one that has been worked out in the study of the nutritive relations of the fungus (4). It is suitable for the growth of *Phymatotrichum omnivorum* but is not so favorable a substratum as to obscure completely the effect of additions of plant juices. It contains per liter: dextrose, 40 gm.; ammonium nitrate, 1.18 gm.; di-potassium phosphate, 1.35 gm.; magnesium sulphate, 0.75 gm.; potassium chloride, 0.15 gm.; and ferric chloride, 0.25 cc. of 0.5 per cent solution. This medium was used at pH 7.0, any adjustment necessary either for the medium alone or with added plant juices being made with potassium hydroxide solution.

Most of the cultures were in 250 cc. Erlenmeyer flasks, 50 cc. per flask, with 6 to 10 replications of each substratum. It was found in the course of the work on the nutrition of *Phymatotrichum omnivorum* that in some media the fungus would develop more rapidly than in others, as well as to a greater final weight; and also that in some media the weight reached a maximum possibly in 3 weeks, decreasing thereafter, while in other media the maximum might not be reached until 5 weeks or more after the date of inoculation. Accordingly, wherever possible, the cultures were run with 10 flasks of each medium, harvesting sets in triplicate, at the ends of 3 and 4 weeks, and in quadruplicate at the end of 5 weeks.

All cultures during the entire experiment were inoculated with *Phymatotrichum omnivorum* strain 24, the same strain that was used for the nutrition studies (3, 4), and inoculum for each series was taken from the sclerotia produced in a single flask of synthetic media. The masses of

sclerotia were pulled out of the flask by needle and placed in a sterile Petri dish on moist filter-paper. Here, a sterile scalpel was used to cut the mass into small pieces. These pieces varied in weight from about 0.15 mgm. to 0.7 mgm., the average size used for inoculum weighing about 0.4 mgm.. oven-dry weight. These weights were negligible as compared to the amount of nutrients ordinarily present in the 50 cc. of solution in the individual cultures, and there was not sufficient material in these sclerotial inocula to support growth of the fungus in distilled water. Individual bits of sclerotia were transferred to the flasks by means of a flat-tip needle, observing the customary aseptic precautions. By this procedure, it was possible to inoculate an entire series of as many as 200 flasks with sclerotia from a single flask. Contaminations were quite rare, and all contaminated cultures were discarded from the series. All the series were incubated in a large culture room, in which during the winter the temperature was held near 26-28° C. by manipulation of heat and ventilation, while in summer it rose to 31-33° C. The results within a given series are thus directly comparable. but this is not true for results in different series.

After the specified periods of incubation, the results were obtained by weighing the mass of fungus growth obtained in each culture. In earlier series each culture was filtered through an asbestos mat in a Gooch crucible, and in later series through alundum crucibles. Care was taken to wash any adhering strands from the flask into the corresponding crucible. The crucibles were dried in the oven at 90–95° C. for 18 hours, to obtain the ovendry weight. The weights of the triplicate or quadruplicate individual colonies determined in this way were averaged to obtain the average dry weight of colonies as listed in the tables below.

EXPERIMENTAL RESULTS

Cotton and corn-root juices. Preliminary comparison of the growth of the root-rot fungus in juices from cotton roots and from corn roots was started with material prepared on September 26, 1930. Portions of the expressed juices were ultrafiltered through Berkefeld filters, and the remainder used in the preparation of autoclaved media. On account of the small volume of juices available, only dilute media were prepared for this series, each juice being used in dilutions with distilled water and with nutrient solution 70, respectively, to 1/10 and to 1/100 of the original concentration.

Growth in the autoclaved juices was only slightly greater than in the checks (Table 1). However, there was definite, although slight, growth in the dilutions with distilled water, the growth in the diluted cotton juice averaging twice the weight of the growth in the corresponding corn-root juice, while there was no growth in the plain distilled water checks. In

TABLE 1.—Preliminary comparison of the growth of Phymatotrichum omnivorum in juices expressed Sept. 26, 1930, from the roots of cotton plants (susceptible) and corn plants (resistant) [5 flasks per series, 50 cc. of medium per flask; incubated at 25-32° C., mean = 27.6° C.]

C	Average dr colon	y weight of <i>P. on</i> ies after 38 days	nnivorum , in
Composition of media	Cotton-root juice	Corn-root juice	Checks
7.2.2.2.2.2.2.2.2.2.2.2.2.2.2.2.2.2.2.2	Mgm.	Mgm.	Mgm.
Autoclaved juices, diluted with distilled	00.0	7900	
water to 0.1	32.0 3.8	$\begin{array}{c} 15.7 \\ 2.3 \end{array}$	
Checks, distilled water alone			0
Autoclaved juices, diluted with nutrient			
solution 70 to 0.1	449.1 442.2	435.0 463.5	***********
Checks, nutrient solution 70 alone	The state of the s		382.8
Ultrafiltered juices, diluted with dis-			
tilled water to 0.1	0 0	0	***************************************
0.01	0	0	
Checks, distilled water alone			0
Ultrafiltered juices, diluted with nutrient			
solution 70 to 0.1	0	0	
0.01	249.4a	0	
Checks, nutrient solution 70 alone			438.6

a Average of growth in 3 flasks, none in others of this series.

ultrafiltered juices, there was no growth except for a few flasks in 1 series of the cotton juice. This inhibition of growth in the ultrafiltered juices has not yet been checked by repeating this series. However, on October 22, a few culture tubes were prepared with ultrafiltered juices of corn and of cotton roots. These juices were used full strength and hence at 10 times the strongest concentration used in the previous series. The tubes were inoculated with sclerotia of *Phymatotrichum omnivorum*, and within 2 weeks a thick mat of typical Phymatotrichum growth was obtained in every tube of cotton juice. Of the 8 tubes with corn juice, very sparse growth appeared in 1 tube and no growth at all in the remainder.

On November 15, 1930, another series of juices was prepared from cotton and corn roots. These juices were sterilized by autoclaving and

were used to prepare the series of cultures listed in table 2. The fungus colonies were weighed after 30-days' incubation. Growth in the undiluted cotton juice averaged nearly 5 times the weight of growth in the corn juice, and in the juices diluted with distilled water growth was greater in the cotton than in the corn juice, even though the total growth obtained was much less than in the undiluted juice.

Some of the juices prepared for the series above were utilized to set up a further series outlined in table 3. This experiment included a number of series with the juices diluted to 1/6 of the original concentration but with the diluents used in the respective series ranging from the complete nutrient solution 70 down to distilled water. Total growth of the fungus was again greatest in the undiluted juice from the susceptible cotton roots. In each of the 8 diluted series, as well as in the original

TABLE 2.—Second comparison of growth of Phymatotrichum omnivorum in autoclaved juices expressed Nov. 15, 1930, from roots of cotton and of corn plants, respectively [5 flasks per series, 20 cc. of medium per flask; incubated at 25-31° C., mean = 28.2° C.]

Composition of substrata	P. omnivor	y weight of um colonies days, in
	Cotton-root juice	Corn-root juice
Undiluted, autoclaved juices	Mgm. 455.5	Mgm. 98.8
Autoclaved juices, diluted with distilled water to 0.1	34.2	5.7
Autoclaved juices, diluted with distilled water to 0.01	2.8	2.5

juices, the growth in the cotton juice averaged a definitely greater weight than the growth in the corn juice. It is to be noted that the growth in the undiluted juices in this series corresponded very closely with the growth obtained in the same juices in the previous series (Table 2).

The fact that the juice from the susceptible cotton roots afforded so much better a substratum for the growth of *Phymatotrichum omnivorum* than did the juice from resistant corn roots suggested that the differences in susceptibility of these plants to root rot might be due in part to the presence in the plants of the materials causing these differences in the nutrient value of the juices. The consistent differentiation shown in table 3 indicates that the difference between the juices probably was not a matter of the presence or absence of one or more of the more common nutrient materials. Since, however, the juice expressed from cotton roots was con-

siderably more concentrated than the juice from corn roots (Table 9), it was considered particularly desirable to extend these studies to juices from other plants between which there was less difference in total concentration of the juices.

TABLE 3.—Growth of Phymatotrichum omnivorum in autoclaved juices expressed from cotton (susceptible) and corn (resistant) roots, diluted in complete and in variously incomplete synthetic solutions [3 flasks per series, 20 cc. of medium per flask; incubated at 26-30.5° C., mean = 28.3° C.]

Composition of substrata	Average dry weight colonies after	of P. omnivorum 30 days, in
	Cotton-root juice	Corn-root juice
	Mgm.	Mgm.
Undiluted juices	429.4	78.2
Juices diluted to 1/6 original concentration,		
with complete nutrient solutiona	335.6	193.0
with nutrient solution complete except for		
dextrose	59.1	25.4
with nutrient solution complete except for		
ammonium nitrate	316.5	173.6
with nutrient solution lacking both dex-		
trose and ammonium nitrate	51.5	14.4
with nutrient solution containing only dex-		
trose	300.3	165.6
with nutrient solution containing only am-		
monium nitrate	47.5	11.2
with nutrient solution containing only		
ammonium nitrate and dextrose	296.7	208.2
with distilled water	56.2	12.1

a	Complete'	nutrient	solution	(No. 70)	contains pe	er liter:	dextrose	40 gr	n.
	Walian Barrier				الزلال وأحيكا	134-41. 495	NH_4NO_3	1.18	
							K2HPO4	1.35	
							MgSO ₄ ·7H ₂ O	0.75	
							KCl	0.15	
	8						FeCl.	trace	

Carrot and onion juices. The fleshy roots of carrots are highly susceptible to root rot, both to natural infection in the field and to laboratory inoculations; while the fleshy bulbs of onions appear completely immune from the disease, even when the fungus is introduced artificially through punctures. These storage bodies therefore furnished a convenient source of material for further tests of expressed juices from susceptible and resistant plants. About 12 lbs., each, of carrots and of yellow onions secured from a local grocery store were used to prepare the juices utilized in the cultures listed in tables 4 and 5. The juices obtained, after autoclav-

ing and refiltering, were found very similar in composition. The total percentage of solid matter in the carrot juice was 6.84 per cent as compared with 5.35 per cent in the onion juice; the two liquids, however, contained almost precisely the same amount of total sugars and exactly the same percentage of total proteins, the chief apparent difference being the 0.59 per cent of ash in the carrot juice as compared with 0.25 per cent in the onion juice (Table 9).

Very definite differentiation of growth in these juices was obtained in the series of cultures summarized in table 4. The greatest growth obtained was

TABLE 4.—Comparison of growth of Phymatotrichum omnivorum in autoclaved juices expressed from carrots (susceptible) and onions (resistant) [7 flasks per series, 50 cc. of medium per flask; incubated at 25-31.5° C., mean = 28.2° C.]

	Incuba-		ry weight oum colonies	
Composition of substrata	tion period	Carrot juice	Onion juice	Checks
Undiluted juices	3 weeks	Mgm. 694.3	Mgm. 265.2a	Mgm.
	5 ''	859.4	0	
Juices diluted with distilled water to 0.25	3 weeks	255.9	226.8	
	5 ''	234.6	196.5	
to 0.1	3 weeks	156.7	120.5	
	5 "	95.1	75.3	
Checks, distilled water alone	3 weeks			0
	5 ''			0
Juices diluted with nutrient solution 70				
to 0.25	3 weeks	596.0	398.4	
	5 "	569.3	577.1	
to 0.1	3 weeks	550.0	369.0	
	5 "	518.2	539.1	
Checks, nutrient solution 70 alone	3 weeks			97.1
	5 "			141.4

a Results in onion juice erratic, good growth in only 1 flask of the series.

in the undiluted carrot juice. In the undiluted onion juice, good growth was obtained in only 1 flask. Such irregularity in growth in the cultures, while characteristic of the onion juice, was unusual in other media, the growth in the replicate flasks usually coming close to the average. For in-

stance, in the 4 flasks of undiluted carrot juice harvested after 5 weeks, the actual weights of total dry matter ranged from 829.9 mgm. to 884.4 mgm., with the average at 859.4 mgm., as shown.

Differentiation was less pronounced in the diluted juices. Growth in the carrot juice diluted with distilled water was consistently greater than in the corresponding onion series, but the difference was not enough to be of much significance. In the dilutions with nutrient solution 70, growth in the carrot juice was significantly greater than in the diluted onion juice at the end of 3 weeks, but the difference was lowered by the continued, although delayed, growth in the onion juice during the next 2 weeks. In both series, the growth obtained was much greater than in the plain nutrient solution.

The following points appear of particular importance: Growth was encouraged by the carrot juice as compared with the onion juice; growth was inhibited in most cases in the undiluted onion juice but not in the onion juice diluted either with distilled water or nutrient solution; and both the carrot juice and the onion juice added materials that greatly favored growth when added to the synthetic nutrient solution.

Further comparison of carrot and onion juice has been obtained in the course of fractionation studies. Since this work is as yet incomplete, only the check cultures, which are directly comparable to the series just described, will be considered here. These cultures are summarized in table 5,

TABLE 5.—Growth of Phymatotrichum in autoclaved juices expressed from carrots (susceptible) and onions (resistant) [8 flasks per series, 50 cc. of medium per flask; incubated at 22-32° C., mean = 27.4° C.]

	Incuba-	Average dry weight of P. omnivorum colonies, in			
Composition of substrata	tion period	Carrot juice	Onion juice	Checks	
		Mgm.	Mgm.	Mgm.	
Undiluted juices	3 weeks	519.7	0		
	4 ''	912.0	0		
	5 ''	1007.7	0		
Juices diluted with nutrient solution 70 to					
0.25	3 weeks	477.1	445.3		
	4 "	684.7	629.6		
	5 ''	710.0	735.9		
Checks, solution 70 alone	3 weeks			107.5	
	4 ''			210.4	
	5 ''			323.1	

which includes results from cultures made with the remainder of the juices that were used in the previous work, and in table 6, which gives some results with a second series of juices prepared on June 3, 1931. The results are almost identical with those presented in table 4. Heavy growth was invariably obtained in the undiluted carrot juice. On the other hand, growth occurred in only 1 of the 20 cultures in undiluted onion juice (in this case from white onions) that were included in these series. Juices from white and yellow onions appear equally toxic to *Phymatotrichum omnivorum*. Dilutions of juices with distilled water allowed growth to occur in the onion juice, and this was true also of dilution with the synthetic nutrient solution. Addition to the undiluted juice of the nu-

TABLE 6.—Growth of Phymatotrichum omnivorum in autoclaved juices expressed from carrots (susceptible) and onions (resistant) [10 flasks per series, 50 cc. per flask; incubated at 28.5-35° C., mean = 32.3° C.]

Communition of substants	Incuba- tion	Average dry weight of P. omnivorum colonies, in			
Composition of substrata	period	Carrot juice	Onion juice	Checks	
		Mgm.	Mgm.	Mgm.	
Undiluted juices	3 weeks	1013.2	0	***************************************	
	4 "	1002.6	159,3a	***********	
	5 ''	802.0	0	•	
Undiluted juices, combined with nutrients	la a la de pla				
of solution 70	3 weeks	1068.2	297.0ь		
	4 "	678.3	309.8℃		
	5 "	1165.7	846.3d		
Juices diluted with distilled water to 0.25	3 weeks	268.3	338.1	•	
	4 ''	235.3	301.4		
	5 ''	188.1	266.4	***************************************	
Juices diluted in nutrient solution 70 to 0.25e	3 weeks	685.9	657.1		
	4 "	800.8	954.5		
	5 ''	613.4	861.2		
보면 함께 된다. 그 보고 이 보면 되었다. 어떻게		11 47 44 4			
Checks, nutrient solution 70 alone	3 weeks			174.7	
	4 "			303.7	
	5 ''			360.0	

a Series variable, colony in one flask only, weighing 478 mgm.

b Weights of individual colonies ranged from 148 to 490 mgm.

d " " " " " " " " " 19 " 1301

e Nutrient solution 70 undiluted in this series.

trient materials used in solution 70 apparently also counteracted in some way the toxic properties of the undiluted onion juice (Table 6).

Taken together, these results suggested that juice expressed from onions, which are resistant to root rot, contains some substance or substances that at the original concentration of the juice prevent growth of the root-rot fungus, while juice expressed from carrots, which are susceptible to root rot, does not contain this property.

Turnip and canna juices. On February 26, 1931, juice was expressed from stored turnips, and from canna roots freshly dug from the ground. Cultures were prepared from autoclaved series as indicated in table 7.

TABLE 7.—Growth of Phymatotrichum omnivorum in autoclaved juices expressed from turnips (susceptible) and canna roots (resistant) [6 flasks per series, 50 cc. of medium per flask; incubated at 22-32° C., mean = 27.4° C.]

	Incuba-	Average dry weight of P. omnivorum colonies, in			
Composition of substrata	tion period	Turnip juice	Canna- root juice	Checks	
Undiluted juices	3 weeks	Mgm. 0 0	Mgm. 0 54.7	Mgm.	
Juices diluted with distilled water to 0.25	3 weeks	167.8 102.2	87.7 52.0	***************************************	
Juices diluted with solution 70 to 0.25	3 weeks 5 ''	689.3 544.3	333.6 469.1		
Checks, solution 70 alone	3 weeks 5 ''			188.1 461.0	

This series is the only one that has been tried in which undiluted juice from a susceptible plant (turnip) has inhibited growth of the fungus. Some slight growth occurred in the undiluted juice from the canna roots. On the other hand, fairly definite differentiation was obtained with the diluted juices. With both diluents, the turnip juice accelerated growth, which was higher at the end of 3 weeks than at the end of 5 weeks and was apparently significantly heavier than the growth obtained in dilutions of the canna-root juice.

Sweet potato and nut-grass juices. On April 23, 1931, juices were expressed from commercial stored sweet potatoes and from the fleshy "nuts," or tubers, together with attached stolons and roots, of the sedge commonly called nut grass, Cyperus rotundus L. These juices were used to prepare

the series of cultures summarized in table 8. Heavy growth was obtained in the undiluted sweet-potato juice but none in that of the nut grass. As usual, the fungus grew in the dilutions of the material. No significant differences appeared between the growth in the various dilutions of the

TABLE 8.—Growth of Phymatotrichum in autoclaved juices expressed from sweet potatoes (susceptible) and nut-grass roots, stolons, and tubers (resistant)

[10 flasks per series, 50 cc. of medium per flask; incubated
at 24-29.5° C., mean = 26.3° C.]

	Incuba-	Average dry weight of P. omnivorum colonies, in			
Composition of substrata	tion period	Sweet- potato juice	Nut- grass juice	Checks	
		Mgm.	Mgm.	Mgm.	
Undiluted juices	3 weeks	306.0	0		
	4 ''	473.3	0		
	5 ''	947.4	0	•	
Juices diluted with distilled water to 0.25	3 weeks	170.4	297.2		
그 집중 집에 불교하는 회에 하였다는 이 나는 그림.	4 "	321.6	344.3		
	5 "	396.8	326.9		
to 0.1	3 weeks	106.7	146.6		
	4 "	181.9	134.3		
	5 ''	195.2	112.1		
Juices diluted with nutrient solution 70 to					
0.25	3 weeks	241.0	332.4		
	4	479.0	730.5		
	5 "	705.8	866.8	•	
to 0.1	3 weeks	153.3	341.9		
	4 "	387.3	615.2		
	5 "	589.2	680.3		
Checks, solution 70 alone	2			3354	
Shocks, Solution to affile	3 weeks			115.4	
	5 "			202.0	
	J			338.7	

sweet potato and nut-grass juices, although it appears that with dilutions in distilled water growth was slower but continued to a greater amount in the sweet-potato juice; while in dilutions in the nutrient solution heavier growth was obtained with the nut-grass juice.

Composition of expressed autoclaved plant juices. Partial analyses of the plant juices tested in these cultures are given in table 9. It will be noted that these analyses throw little light on the differences between the juices that may be involved in the relative value of the juices as substrata for *Phymatotrichum omnivorum*. It is possibly of some importance that each of the 4 juices from plants susceptible to root rot was more concentrated than the juice from the resistant monocotyledonous plant with which

TABLE 9.—Composition of various plant juices used in these experiments; from analyses of autoclaved, refiltered, and re-autoclaved juices, prepared exactly as for culture substrata

	Tables in	les in Compositio			n, as gm. per 100 cc.			
Sources of juices	which cul- ture results are given	рH	Total dry matter	Total sugars	Reducing sugars	Proteins (N x 6.25)	Ash	
Cotton roots (susceptible)	Tables	6.0	5.86				0.94	
Corn roots (resistant)	2 & 3	6.0	2.42				0.49	
Carrots (susceptible)	Tables	5.7	6.84	4.67	2.43	0.63	0.59	
Onions (resistant)	4 & 5	5.4	5.35	4.57	3.84	0.63	0.25	
Turnips (susceptible)	Table	5.8	4.00	2.60	2.46		0.58	
Canna roots (resistant)	7	6.1	3.11	1.24	0.52		0.90	
Sweet potatoes (susceptible)	Table	5.7	8.49	5.77	1.55	0.18	0.44	
Nut grass (resistant)	8	5.4	7.91	3.37	0.69	0.55	1.26	

it was compared. Yet, the nut-grass juice, in which no Phymatotrichum growth occurred, was more concentrated than any of the juices from the susceptible plants except the sweet-potato juice. There was no consistent difference between the juices with regard to the pH, the total nitrogen content, the total ash content, or the particular constituents of the ash so far as analyzed.

DISCUSSION

These studies of the growth of the root-rot fungus in juices expressed from the underground parts of plants included material from 4 dicotyle-donous plants that are susceptible to root rot, as compared with material from 4 monocotyledonous plants that are resistant to the disease. The juice extracted from every susceptible plant except the turnip proved a very good substratum for the fungus, which developed a much greater weight of mycelium than was ever obtained either on the check synthetic solution or in the juice from the resistant plants. Consistently poor growth was secured in the undiluted juices from the 4 monocotyledonous, resistant plants that were included in the study, except for the occasional erratic growth in the undiluted onion extract. Dilution of the juices from sus-

TABLE 10.—Summary of growth obtained in juices expressed from susceptible plants (upper of each pair) and from resistant plants (lower of each pair) in the undiluted, autoclaved juice and in juice diluted to \(\frac{1}{2}\) its original concentration with distilled water [See original tables for details about various series]

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	Reference	Average dry weight of Phymatotri- chum omnivorum colonies, in			
Sources of juices	to table	Undiluted juices	Juices diluted to ‡ with distilled water		
Cotton roots (susceptible) Corn roots (resistant)	Table 2	<i>Mgm</i> . 455.5 98.8	Mgm. 		
Cotton roots (susceptible) Corn roots (resistant)	Table 3	429.4 78.2			
Carrots (susceptible) Onions (resistant)	Table 4	859.4 265.2	255.9 226.8		
Carrots (susceptible) Onions (resistant)	Table 5	1007.7 0			
Carrots (susceptible) Onions (resistant)	Table 6	1013.2 159.3	268.3 338.1		
Turnips (susceptible) Canna roots (resistant)	Table 7	0 54.7	167.8 87.7		
Sweet potatoes (susceptible) Nut grass (resistant)	Table 8	947.4	396.8 344.3		

ceptible plants with distilled water resulted invariably in lessening the growth of the fungus, except in the case of the turnip juice. On the other hand, dilution of the juice from resistant plants led usually to a higher average weight, due to the less erratic growth in these diluted liquids. Thus, there was marked differentiation in the relative growth in undiluted juices from the susceptible and resistant plants, but less differentiation in the growth in the diluted juices (Table 10).

The number of plants included in these studies was sufficient to suggest that the differences observed in the nutritive value of the plant juices to the root-rot fungus is perhaps connected with the differences in the resistance and susceptibility of these plants to Phymatotrichum root rot. Susceptibility or resistance to root rot in these cases would then be classified as physiologic and of a nature comparable to that suggested for onion smudge (15), flax wilt (10), and stem rust of wheat (2).

Further study to find, if possible, the nature of the substance or substances involved in this reaction of plant juices to the growth of the root-rot fungus is now under way, and the results will be reported later. For the present, from the fact that the undiluted juice from resistant plants partly or entirely inhibited the growth of the root-rot fungus, while, after dilution with distilled water, this inhibition appeared to be mostly removed, we may draw the tentative conclusion that the difference between the 2 types of plants can scarcely involve lack of essential nutritive material in the resistant plants. The difference would, instead, appear to involve the presence of some specifically inhibitory material in the resistant plants.

SUMMARY

- 1. Phymatotrichum omnivorum, the fungus that causes root rot, has been grown in series of cultures prepared with juices expressed from 4 monocotyledonous plants (corn, onions, cannas, and nut grass) resistant to the disease and from 4 dicotyledonous plants (cotton, carrots, turnips, and sweet potatoes) susceptible to the disease. The oven-dry weight of the mycelium was determined.
- 2. Growth of the fungus was markedly inhibited in the undiluted, though autoclaved, juices from all of the resistant plants, while profuse and heavy growth was obtained with juices from 3 of the 4 susceptible plants.
- 3. With diluted juices, good growth was obtained even in series from the resistant plants.
- 4. It is therefore concluded that monocotyledonous plants resistant to root rot apparently contain materials that in sufficiently high concentration can inhibit the growth of the root-rot fungus, and that the resistance of these plants to the disease is probably based, at least in part, on the presence of this material or materials. Further study is under way to determine more accurately the materials involved.

COLLEGE STATION,

TEXAS.

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REPORT OF THE TWENTY-THIRD ANNUAL MEETING OF THE AMERICAN PHYTOPATHOLOGICAL SOCIETY

THE NEW ORLEANS MEETING

The American Phytopathological Society held its twenty-third annual meeting from Tuesday through Thursday, December 29-31, 1931, in conjunction with its southern division, with about 125 pathologists in attendance. Excellent space for meetings was provided by the St. Charles Hotel (headquarters) and Tulane University.

Abstracts of the 89 papers delivered before the Society's several sessions were published in the January, 1932, number of PHYTOPATHOLOGY. These papers may be grouped as follows: General (including two invitation papers), 3; potato diseases, 5; fruit diseases, 12; biologic specialization, 8; tobacco and sugar-cane diseases, 8; vegetable diseases, 16; mycology, 8; trees and ornamentals, 9; cereal diseases, 8; corn diseases, 8; miscellaneous crop diseases, 4.

Three joint sessions were held as follows: with Section G of the A. A. A. S., Potato Association of America, and the Mycology Section of the Botanical Society of America.

Extension work in plant pathology, with special reference to southern extension problems in the light of present conditions, was the subject outlined for discussion at the meeting of the extension pathologists on the afternoon of December 29. The importance of reviewing and revising programs of work to see that they meet the needs and are economically sound and in line with the State programs was stressed, as was also cooperation with other specialists in conducting the work. The conference was informed of a method of conducting demonstrations on a large scale in Iowa. Contests for large yields of various crops, as illustrated by "400-Bushel Potato Clubs," were considered. Among other subjects discussed were: Zinc-lime sprays for peaches; dipping sweet-potato sprouts; need of a standard method of making Bordeaux mixture; control of cucumber and watermelon diseases; and need of revision of standards for certifying Irish potatoes.

In a round-table session on quarantine and regulatory work discussion of "Nematodes as a Quarantine Problem" brought out vividly the meagerness of our knowledge of the pest status of these organisms, and the views expressed on "Uniformity in Nursery Inspection" stressed the general need of such uniformity to be best secured by interstate agreement arrived at through the plant-board system.

The phytopathologists' dinner, with 143 attending, was a great success. Under the leadership of Dr. C. H. Edgerton a committee of plant pathologists from the Southern States provided musical entertainment by students of Southern University, and other much appreciated specialties.

OFFICERS AND REPRESENTATIVES

The following officers and representatives were chosen:

President, F. D. Heald, State College of Washington, Pullman, Wash.

Vice President, J. G. Dickson, University of Wisconsin, Madison, Wis.

Secretary-Treasurer (three years), F. C. Meier, U. S. Department of Agriculture, Washington, D. C.

Councilor (two years), L. O. Kunkel, Boyce Thompson Institute for Plant Research, Inc., Yonkers, N. Y.

Editors (three years), H. B. Humphrey, Editor in Chief, U. S. Department of Agriculture, Washington, D. C.; H. M. Quanjer, Editor for Europe, Instituut voor Phytopathologie, Wageningen, Holland; Annie Rathbun Gravatt, U. S. Department of Agricul-

ture, Washington, D. C.; and Eubanks Carsner, Citrus Experiment Station, Riverside, Calif.

Associate Editors (three years), J. C. Gilman, Iowa State College, Ames, Iowa; W. D. Valleau, University of Kentucky, Lexington, Ky.; W. F. Hanna, Manitoba Agricultural College, Winnipeg, Canada; and Jessie I. Wood, U. S. Department of Agriculture, Washington, D. C.

Business Manager (one year), F. C. Meier, U. S. Department of Agriculture, Washington, D. C.

Advertising Manager (one year), R. S. Kirby, Pennsylvania State College, State College, Pa.

Representatives on the Council of the American Association for the Advancement of Science (one year), D. Reddick, Cornell University, Ithaca, N. Y.; and C. W. Edgerton, Louisiana Agricultural Experiment station, Baton Rouge, La.

Member of the Board of Governors of the Crop Protection Institute (three years), W. H. Martin, New Jersey State Agricultural Experiment Station, New Brunswick, N. J.

The following temporary committees were appointed to serve throughout the meetings:

Auditing Committee, H. A. Edson and J. F. Adams.

Committee on Elections, H. T. Cook, Duke Layton, and H. N. Hansen.

Committee on Resolutions, H. W. Anderson, E. C. Stakman, and G. F. Weber.

REPORT OF THE SECRETARY-TREASURER, 1931

The final check up of membership last year showed a total of 753. Eighty-seven new members elected at the Cleveland Meeting and 3 former members reinstated during the year brought this number to 843. During 1931, 4 members were lost through death, 8 resigned, and 43 were dropped from the Society for nonpayment of dues. The total membership, therefore, now stands at 788. Of this number we have 85 paid-up life members, 89 life-sustaining, and 614 regular members. There are 48 new applicants for membership. Election of these will give us a total of 836.

STATEMENT OF ACCOUNTS FOR 1931, AS OF DECEMBER 15, 1931

Receipts:	
Balance from 1930	\$3,182.07
Annual dues: 1930 \$ 20.00	
1931 1,709.55 (\$260.25 life)	
1932	
1933	3,945.42
Interest on checking account	43.55
Donations to Lyman Memorial Fund included in checks for dues	22.00
Donations to Butler Fund	139.32
Sales received with dues	19.20
Subscription received with dues	6.52
Refund on advance of funds to Secretary-Treasurer for Cleve-	
land meeting	3.96
Balance from Phytopathologists' dinner in Cleveland	43.00
Total Receipts	\$7,405.04
Expenditures:	
Member subscriptions transferred to PHYTOPATHOLOGY	
(1931, part 1930)	\$3,688,24

Secretarial work	455.53
Printing (programs for 1930 and 1931, preliminary programs,	±00.00
abstracts, special notices, application blanks, etc.)	215.55
Advance of funds to Secretary-Treasurer for expenses of Cleve-	210.00
land meeting	75.00
Butler settee	91.68
Postage and stamped envelopes	83.49
Donations transferred to Lyman Memorial Fund	22.00
Multigraphing 1930 press releases	15.00
Telephone and telegraph	7.21
Checks returned by bank	30.00
Furniture and supplies	47.30
Sales transferred to PHYTOPATHOLOGY	15.00
Subscription transferred to PHYTOPATHOLOGY	6.52
Domestic mailing list	1.31
Balance on hand	2,651.21

\$7,405.04

SINKING FUND

The Sinking Fund, obtained by deducting \$5.00 (formerly \$6.00) from each life-sustaining-membership payment, is now \$7,236.17, of which \$6,500.00 is invested in first-mortgage notes. The remaining \$736.17 is now in the Society's checking account and will be invested as recommended by the investment committee.

Respectfully submitted,

F. C. MEIER, Secretary-Treasurer.

REPORT OF THE BUSINESS MANAGER OF PHYTOPATHOLOGY FOR 1931 STATEMENT OF ACCOUNTS FOR 1931, AS OF DECEMBER 15, 1931

eceipts:	A1 000 6
	\$1,083.
Subscriptions, 1931	2,579.
Subscriptions, 1932	748.
Subscriptions, 1931 Subscriptions, 1932 Subscriptions, other years	36.
Sales of back volumes and numbers	233.
Advertising, 1930	76.
Advertising, 1931	795.
Advertising, 1931 Interest on Sinking Fund	390.
Member subscriptions for 1930 (balance)	786.
Member subscriptions for 1931 (part)	
Member subscriptions (balance earlier years)	44.
Excess payment on group of subscriptions	26.
Dues to The American Phytopathological Society received with	
subscriptions	5.
Tropical Plant Research Foundation for color plate for Priode	
article	377
For special notice in July article	3.
Refund on insurance of old volumes	12

Expenditures:

Manufacturing PHYTOPATHOLOGY:	
Vol. XX, No. 12	
Vol. XX, Index	
	\$ 625.04
Vol. XXI, No. 1	735.00
No. 2	581.17
No. 3	554.67
No. 4	559.18
No. 5	
No. 6	355.94
No. 7	687.99
No. 8	420.24
No. 9	429.91
No. 10	419.24
No. 11	495.45
Vol. XXI, Cuts	
Color plate	
Postage	429.43 7,634.78 8,259.82
Secretarial work for Business Manager	
Expenses of Editor in Chief	67.53
Postage and envelopes	
Expenses of Advertising Manager	128.57
Refund of excess payment	26.00
Transferred to The American Phytopathologic	cal Society for dues 5.00
Refund to Bayer Semesan Co. for patron me	
Refund on sales	1.00
Reprints and mailing lists	
Insurance on early volumes	
Inventory of early volume	3.33
Expenses of Business Manager and Advert	ising Manager on
trip to Lancaster, Pa.	16.60
Supplies	
Balance on hand	1,227.33

There were 565 subscribers (nonmembers) to PHYTOPATHOLOGY in 1931. With 6 complimentary subscriptions and 788 members of The American Phytopathological Society, this brings the total circulation of the Journal to 1,359.

To Dr. J. F. Adams, who is retiring after a period of 8 years' successful service as advertising manager, the Society owes much. From the beginning his energetic efforts to build up advertising as a source of income brought results that have enabled the Society to print a better journal.

Recognition should also be made of the service rendered by Dr. C. E. Temple, of Maryland, who for a number of years has voluntarily handled the distribution of back numbers of the Journal as printed by Williams & Wilkins; also to the University of Maryland, which has provided storage for this stock.

To our editor in chief, Dr. H. B. Humphrey, who often against his personal inclination has resolutely limited the number of printed pages during the year, we owe the fact that the sound program for increased income initiated during the term of Dr. R. J.

Haskell, my predecessor, has made it possible for the Journal to attain a much improved financial footing.

Respectfully submitted,

F. C. Meier, Business Manager.

REPORT OF THE EDITOR IN CHIEF

The consistent improvement and maintenance of the standards of excellence of our Journal have been live subjects of interest and thought in the mind of each member of the editorial staff. Many have been the expressions, both written and spoken, testifying to the fact that PHYTOPATHOLOGY has from month to month reflected not only the solicitude of its editors but also the awakened intelligent interest of its contributors and readers. It would seem that more than ever is there evidence of the fact that the improvement and increased usefulness of our Journal are a responsibility resting not alone upon the editors but, in a very real sense, upon The American Phytopathological Society as a whole.

We realize that there yet remains no little need of further improvement. It may be that purely voluntary, nonrenumerated editorial service will never bring about the editorial finish that characterizes certain other scientific periodicals. Nevertheless, "the game is worth the candle." With the continued effort on the part of each contributor to organize his subject-matter and to polish off the finished product in the form of readable, lucid, and scientifically excellent manuscripts we can and inevitably must raise the standard of PHYTOPATHOLOGY.

It still seems necessary to admonish some of our contributors to devote more attention to the art of science writing. Prolixity is inexcusable. To express in 30 words a fact or thought requiring but 10 is an indefensible waste of time and money. Publication of the current volume of our Journal has cost the Society \$7,888.88, or \$6.53 per page. It is very probable that we paid too dearly for the actual subject-matter presented in the more than 1,200 pages. When we prepare a manuscript let us assure ourselves that those supporting Phytopathology will not have to pay for any "sand-in-the-sugar" contributions.

The 21st volume of Phytopathology comprises 1,207 pages of printed matter and illustrations, classified as follows: 89 articles, 18 phytopathological notes, 3 reports, 6 book reviews, 98 abstracts (2 by title only), 1 color plate, and 256 text figures. During the period January 1 to December 31, 1931, approximately 121 manuscripts of articles, phytopathological notes, reports, book reviews, etc., and 89 manuscripts of abstracts (1 by title only) were submitted. Of this number, 19 manuscripts were returned to the authors for revision and 2 were rejected. Of the several manuscripts received during the current year, 11 articles, 1 phytopathological note, 1 book review, and 89 abstracts (1 by title only) are now in press. Approximately 28 per cent of the papers, exclusive of abstracts, appearing in volume 21 were contributed in part or wholly by employees of the U. S. Department of Agriculture.

Summarized report for the triennium, 1929 to 1931. The 19th, 20th, and 21st volumes of Phytopathology comprise 3,365 pages of printed matter and illustrations, classified as follows: 264 articles, 63 phytopathological notes, 8 reports, 12 book reviews, etc., 357 abstracts (17 by title only), 40 plates, and 640 text figures. During the period January 1, 1929, to December 31, 1931, approximately 385 manuscripts of articles, phytopathological notes, reports, book reviews, etc., and 355 manuscripts of abstracts (18 by title only), exclusive of those rejected, were submitted and presented at the annual meetings of The American Phytopathological Society and the meetings of the Pacific and Canadian divisions of the Society. Of this number, 73 were returned to the authors for

revision, 18 were rejected, and 6 were voluntarily withdrawn. Of the total number of manuscripts received, 48 are now on the printer's waiting list. Approximately $\frac{1}{3}$ of the articles published in volumes 19, 20, and 21 were contributed by employees of the U. S. Department of Agriculture. From sources exclusive of continental United States, there have been received and accepted 73 contributions, 23 of which were submitted from Canada; 14, Hawaii; 5 from each of the following: Great Britain, India, and The Netherlands; 4, Peru; 3, each, Cuba and Porto Rico; 2, each, Palestine and China; and 1, each, from the Philippines, Santo Domingo, Argentina, Colombia, France, Union of South Africa, and Tanganyika, Africa. From these figures and figure 1 it is readily

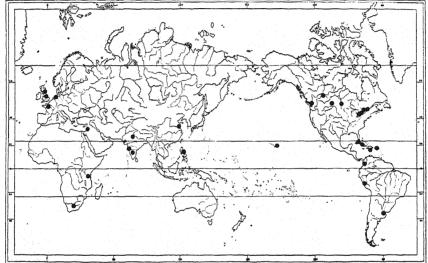


Fig. 1. Mercators'-projection map showing geographical distribution of points of origin, outside of continental United States, of contributions to PHYTO-PATHOLOGY during the triennium 1929-1931

patent that our Journal is, in fact as well as name, international. Publication of an occasional contribution in certain languages other than English would extend its usefulness and enlarge its internationality.

The editor wishes here to make grateful acknowledgment of the unstinted cooperation and exceptional service of the several members of the editorial board. Special thanks are due Mr. J. Marion Shull for his valued help and advice in the arrangement and lettering of illustrations accompanying the several contributions and to Frances W. Todd for invaluable editorial and clerical assistance.

Respectfully submitted,

H. B. HUMPHREY, Editor in Chief.

REPORT OF THE ADVERTISING MANAGER

For the first time in the past 7 years, the total of advertising secured during 1931 is below the average. The previous 5-year average has been 73 pages. The 1931 total dropped to 54% pages. For the 12 issues of Phytopathology published during 1931, there appeared a total of 83 advertisements distributed as follows: 47 one-page, 15 one-half page, and 21 one-fourth page advertisements.

The renewal of contracts for the coming year is always started during the preceding fall, usually in September. Greater solicitation and a broader field had been covered for business during 1932. Replies received indicate further retrenchment in budgets for advertising. In several instances a 50 per cent cut in the budget for 1932 has been made and many replies indicate that curtailment has been so drastic that they regret the possibility that no advertising can be placed for 1932. At this time, the total of advertising secured for 1932 is 60 per cent of the total secured during 1931.

Respectfully submitted,

J. F. ADAMS, Advertising Manager.

COMMITTEE ON INVESTIGATION OF FOREIGN PESTS AND PLANT DISEASES

Your committee begs to call attention to certain plant diseases that have come to our attention during the past year and that are not yet known to occur in this country, but present sufficient danger to our crops to be investigated before they have became introduced.

A particularly outstanding case is the potato rust, *Puccinia pittieriana* until recently known only from Costa Rica and Ecuador where it was very destructive to potatoes and tomatoes. In 1929 it was found in Peru and in 1930 in *Solanum demissum* in Mexico by Dr. Donald Reddick.

The range of Lima-bean scab (*Elsinoë canabaliae*) is now included to cover Mexico, Gautemala, and Costa Rica, in addition to Cuba and Porto Rico where it was previously known. This disease is unquestionably of great importance in Cuba, and information is urgently needed as to its pest status in the United States.

We wish to emphasize the importance of avoiding new importations of pathogenic races of plant parasites. A new race of corn smut is of as much importance pathologically as a new species of smut.

In this connection there seems to be some doubt as to the distinctiveness of the various species of Sphaceloma, at present recognized as causing citrus scab in this country and the West Indies. The exact relationship of these species or races should be established in order that the further importation of citrus may be properly regulated.

C. R. ORTON, Chairman,

W. A. McCubbin,

F. D. FROMME.

COMMITTEE ON QUARANTINE AND REGULATORY WORK

The attendance at the session on Quarantine and Regulatory Work held during the New Orleans meeting was made up as follows: Washington, D. C., 3; Mississippi, 4; Minnesota, 2; and one each from Delaware, Maryland, New Jersey, North Carolina, and Texas—total, 14.

The discussion on "Nematodes as a Quarantine Problem" was ably introduced by R. F. Poole, of North Carolina, who brought up six phases of the subject for consideration, accompanying his presentation with comments designed to bring out the difficulties confronting us in each case. The points presented were as follows:

- (1) Are nemas already so widely distributed that further precautions in dissemination and spread may be of doubtful value?
- (2) Are nemic diseases increasing in importance or have they always been severe but not properly diagnosed?
- (3) Are nemas restricted to southern climatic and soil conditions and controlled by these conditions in northern States.

- (4) Are nematode species sufficiently restricted to crop species that rotation practices can be regulated sufficiently to obtain control or satisfactory reduction in losses?
- (5) Are methods of seed and plant treatment so effective in control that plant quarantine measures are unnecessary?
- (6) Are nemic diseases and methods of diagnosing the nematode species sufficiently accurate and well known to warrant quarantine procedures?

The discussion that followed brought out the attitudes of the various State representatives as to how nemas are regarded as a regulatory problem in each State. The general opinion seemed to be that quarantine or regulatory action would be advisable only in special cases, particularly where enough was known about the nema involved to justify this type of action.

From a number of quarters the complaint was made that too little is known about specific identities, life histories, host range, damage, and control to provide a sound basis for judgment whether regulatory measures would be useful or unprofitable.

Questioning of the State representatives present elicited the fact that in none of these States was any general investigation of the nematode problem in progress, although in some cases attention was being given to specific diseases due to nemas.

Mention was made of the considerable damage on sweet potatoes caused by the bulb nema, and the meeting adopted the following resolution for transmission by the secretary of the Society to the proper Federal authorities:

"That in view of the fact that the bulb nematode (Tylenchus dipsaci) has been reported recently as serious on the sweet potato in New Jersey, and since the possibility exists that this may become a very serious disease of this important crop, it is the opinion of this group that immediate steps be taken to determine the present distribution of the disease. This should no doubt be done through surveys of storage houses this winter and spring, as well as through careful inspection of plant beds where poor germination is reported, since this poor germination is one of the symptoms of the presence of this nema. It is urged also that all available information on this disease of the sweet potato be assembled and placed in the hands of plant pathologists, horticulturists, county agents, plant boards, and all other interested parties."

The second subject for discussion was well introduced by Dr. Clay Lyle, of Mississippi, who pointed out the various difficulties arising from the endless variation in nursery-inspection certificates, requirements, standards, and methods of inspection now in effect in different States; the efforts now being made by regional and national plant boards to bring about more uniformity in these matters; and the responsibility of the plant pathologist in helping to solve the problems involved.

In spite of this very promising beginning the discussion had to be curtailed by shortness of time, so that little of constructive value came out of the session on this subject.

W. A. McCubbin, Chairman.

COMMITTEE ON INTERNATIONAL RELATIONS

Your committee was informed that at a meeting of the General Assembly of the International Union of Biological Sciences held in Brussels in July, 1931, the question of the status of phytopathology in the Union was settled by erecting a subsection for pathology in the botanical section. Dr. Johanna Westerdijk was named chairman of the

subsection. The former president of the Union has stated in a letter (the *Proceedings* are not yet available) that further organization of the subsection will probably await adherence of the principal national societies.

Your committee has examined the statutes of the International Union of Biological Sciences and is of the opinion that the aims and purposes of the Union are substantially in accord with the "principles" adopted by this Society at its Des Moines meeting. Under the very broad and thoroughly international statutes of the International Research Council the Union enjoys a very great freedom of operation amounting practically to autonomy.

The Union is a going concern and the subsection for phytopathology can be made just as active and as useful in the furtherance of international relations as the constitutent national organizations choose to make it.

The following resolutions are, therefore, recommended to the Society for adoption:

- 1. That in view of the prevailing sentiment in the Society for closer international relations as expressed at various times, but in particular at the Des Moines Meeting, The American Phytopathological Society look with favor upon adherence to the International Union of Biological Sciences as at present constituted under the International Research Council.
- 2. That the Council be empowered to institute negotiations looking to adherence to the Union.
- 3. That a copy of this resolution be transmitted to each of the biological organizations now in session in New Orleans.
- 4. That, if the Society adopt the foregoing resolutions, this Committee be discharged.

R. J. HASKELL, Chairman.

PRESS SERVICE

During the past year the Press Service has continued to release for the public press stories based on the papers presented at the annual meetings and to assist the Society in any matters pertaining to its publicity.

Better to serve the Society and to obtain the journalist's view of the material handled, Don B. Reed, day city editor of the Washington *Post*, was taken into consultation. Following Mr. Reed's excellent suggestions, a few of the papers for the New Orleans meetings were given amplification and released as special features, while the remainder were grouped according to crops or groups of crops, covered, and sent out as material for special columns or to be used as "punk", at the discretion of the receiving editor.

Press releases of this nature are very useful in presenting to the public the achievements of plant pathologists, yet it is a rather thankless task since there seldom is any way in which the use of material may be checked. However, from the reports that have filtered in already this year and those of past years, it is most gratifying to the Press Service to report not only a wider dissemination of the material but an apparent increase in the number of papers using it.

Besides the usual press releases for the annual meetings, the Press Service has cooperated with the Secretary-Treasurer of the Society in preparing the report of the meetings published in *Science* and in writing advertisements for the Society and for PHYTOPATHOLOGY.

COMMITTEE ON NECROLOGY

During the year 1930, there was one death not included in the previous report, namely, that of Mr. Sanpatiro Umetu. Mr. Umetu died in Japan in 1930, but it has been impossible to determine the exact date of his death.

During the calendar year 1931, there have been four deaths, as follows:

Mr. Frederick John Pritchard, died January 13, 1931.

Dr. Louis Herman Pammel, died March 23, 1931.

Dr. Warner Jackson Morse, died March 25, 1931.

Dr. Calvin Henry Kauffman, died June 14, 1931.

A. G. Johnson, Chairman,

G. P. CLINTON.

M. B. WAITE.

REPORTS OF OTHER COMMITTEES AND REPRESENTATIVES

Report of Activities of the Division of Biology and Agriculture of the National Research Council. The elector from the Society of American Bacteriologists and I selected Dr. James M. Sherman to represent the two societies.

A. J. RIKER.

Elector Representing The American Phytopathological Society.

The American Association for the Advancement of Science. A brief informal report of the representatives on the Council of the American Association for the Advancement of Science was presented by C. W. Edgerton.

Report on the American Type Culture Collection, 1931. The following is a report on the portion of the American Type Culture Collection maintained by the Division of Mycology and Disease Survey, of the Bureau of Plant Industry, for the year 1931:

Number	of	cultures	sent	to 1	headquarters	in	Chicago	during	the	
year										213
Number	of	cultures	in sto	ock	Dec. 18, 193	30				654
Number	of	cultures :	in stoc	k I	Dec. 8, 1931					784

In addition to the cultures sent to Chicago, approximately 150 cultures have been supplied to departmental investigators and cooperators.

I regret to report that the funds heretofore provided to maintain the collection and carry on the work are almost exhausted, and no more can be obtained from the same or any other source at present known. It was hoped by some of the supporters of the project that it could be made self-supporting, but this does not seem to be possible. Some new method of financing or carrying on the work must be found, if the collection as a whole is to be maintained. If any member of our Society can suggest within the next few months ways or means of continuing the work, such suggestion would be gratefully received.

C. L. SHEAR.

Committee on Chicago Century of Progress Exposition. Dr. L. R. Jones was appointed chairman of this committee but, owing to his absence in the Orient, he deputized H. W. Anderson to act as chairman during his absence. Before leaving, Dr. Jones sent the present chairman some general suggestions in regard to possible methods of interlocking our exhibit with other phases of science, especially the basic science of botany and the applied science of agriculture. I quote his letter to the other members of the committee:

"Or. Woods, as an active member of the Science Advisory Committee, is well informed as to progress to date and is especially pleased at the action of our Society in proffering its services through this Committee. He promises to cooperate by keeping us advised of further developments, will welcome suggestions or general aid, and will look to us for more specific services as plans take definite form. There is now before Congress a bill providing financial aid. He will advise us when this passes as to provision and possibilities under it. Meanwhile he recently sought advice from his departmental and other Committee associates and as a result has prepared certain 'Suggestions for the Agricultural Exhibit.' I am asking him to send a stencil copy of this to each of you for your information. Meanwhile I am inclosing a more general printed pamphlet, which he gave me, for your information.

"Or. Woods expects the Congressional Bill to be acted upon at this session but does not know what limitations may be placed on its use. It is my present conception that the scientific exhibits may take two general forms: (1) A coordinated central group dealing with the Century of Progress in the basic sciences; (2) other related exhibits dealing with the human relations or applications of science. We may assume that mycology, bacteriology, and physiology may receive consideration in the first group, and I believe that the basic concepts of parasitism (the 'germ theory of disease') as related to animal and plant pathology may also deserve consideration. It is in the second group that the applications to plant cultural practices—and probably that which deals with specific diseases as such—may find place.

"While waiting for more specific advice on these points from Dr. Woods, I make the following suggestions and request:

"(1) That Dr. Anderson learn whatever he may, especially through relations with Dr. W. P. Flint (as a member of the Science Advisory Committee), concerning the plans of the entomologists for corresponding exhibits, including what place these may have in the central or basic exhibits. Also whatever may be learned as to financial support expected for such exhibits, including possible State funds.

"(2) That Dr. Link learn whatever he may upon these and related matters, especially through relations with the officials of the central administration in Chicago."

The acting chairman has taken occasion to confer with Dr. Gregory and Dr. Link during the past summer in order to get their viewpoints as to the best method of planning the exhibits.

There seem to be several possible methods of exhibiting the science of plant pathology.

1. The committee might act in an advisory capacity for those in charge of the general agricultural and botanical exhibits.

2. Special funds and space might be secured to be devoted to plant pathology alone.

It seems to be the idea of the central committee of 50 of the Century of Progress that all exhibits should be arranged in such a way as to make a continuous story; in other words, that the specialized sciences be grouped around the basic science to which they are most closely related.

In the case of plant pathology, the difficulty arises from the fact that it may be regarded as a phase of biology or as an applied science in agriculture. It seemed, therefore, that the first question to decide was whether we should ally ourselves with the agricultural group or with the botanical group or both.

Some general plans were suggested by both Dr. Gregory and Dr. Link, but before any very definite plans could be carried out we thought it best to learn something about the financial help we could depend upon to carry out these plans.

Dr. Jones suggested in his letter that the Federal Government planned to make a special appropriation for the exposition, but to date we have had no word from Dr.

Woods. Also, the State of Illinois expected to appropriate money for the exposition, but has not yet done so. Until funds are available, no definite plans can be made.

The acting chairman has been somewhat remiss in that he has not had any extensive correspondence with those in charge of the various phases of the exposition. Before doing this, he has felt that some provision should be made by the Society to have a meeting of its committee in Chicago, where some ideas could be obtained at first hand as to the possibilities of a worth-while exhibit. At least those members close to Chicago could attend this meeting. It is quite difficult to visualize the plan of the exhibition from the general plans that are available at the present time.

H. W. ANDERSON, Acting Chairman.

Committee on Extension Program. A full day's extension program was originally planned for New Orleans. In the course of correspondence it was decided that discussion of successes and failures in projects would bring out all the essential details needed in putting across any projects, probably in a better way than to try to analyze the question and present the different phases of the question.

Among other things we wished to point out how the extension pathologist is constantly discovering new problems for research and how he must modify and change the discoveries of the research men before they became adaptable to farm conditions.

Owing to the inability of many extension pathologists to attend the meetings this year, this program was postponed until another year, and it was agreed to revive the EXTENSION PATHOLOGIST and to carry out our discussions in its pages.

CHAS. GREGORY, Chairman.

Auditing Committee. The accounts of The American Phytopathological Society and of PHYTOPATHOLOGY for the year past have been examined and the totals of accounts for receipts and expenditures, including vouchers, were found satisfactory and correct.

H. A. Edson, J. F. Adams.

Resolutions Committee. A committee consisting of E. C. Stakman, G. F. Weber, and H. W. Anderson, brought in the following report, which was adopted by the Society.

Resolved: That the members of The American Phytopathological Society extend their sincerest thanks to the members of the local committee on arrangements for their efficient efforts in contributing to the success of the New Orleans meeting.

Resolved: That the Society express its appreciation to Tulane University for the excellent facilities furnished for the meetings of the various sections of the Society.

Resolved: That the members of the Society extend their sincere thanks to Prof. C. W. Edgerton and his colleagues for the splendid entertainment furnished those in attendance at the New Orleans meeting.

Resolved: That The American Phytopathological Society express to President C. S. Clark of Southern University their appreciation of the splendid musical program furnished by the male quartet of his institution and their accompanist at the annual banquet of the Society.

ACTION OF THE COUNCIL

In addition to making the appointments mentioned in the first part of this report, the Council reported the following actions which were approved by the Society.

1. On consideration of an invitation to meet with the A. A. A. S. summer meeting, scheduled for Syracuse, N. Y., in late June, it is recommended that no summer meeting be held in 1932.

- 2. That, as was the case in 1930 and 1931, the editor of PHYTOPATHOLOGY be allowed actual expenses for 1932 up to or within \$300.00 for secretarial and editorial assistance.
- 3. That the editor in chief be reimbursed by the Society for his travel expenses to the present meeting.
- 4. That the Business Manager of PHYTOPATHOLOGY, in order to make storage space for the growing supply of back numbers, be authorized to offer for sale during a limited period excess volumes of PHYTOPATHOLOGY down to a limit decided by him as safe, at a price of approximately \$3.00 per volume plus postage.

MISCELLANEOUS BUSINESS

The reports of the various officers and committees as given in the preceding pages of this report were adopted by the Society.

The Secretary read a letter of November 9 from Dr. E. J. Butler, Director of the Imperial Mycological Institute, England, in which reference was made to the settee presented that institution by this Society. The following paragraph from the letter is quoted:

"In conclusion may I ask you to express to the donors our great appreciation of this most graceful and generous mark of their interest in the work of the Imperial Mycological Institute which I hope will always and to an ever-increasing extent serve as one of the links that unites the phytopathologists of the two great English-speaking races."

FREDERICK JOHN PRITCHARD DECEMBER 24, 1874-JANUARY 13, 1931

Frederick John Pritchard was graduated from the University of Nebraska in 1904 with the degree of Bachelor of Science. He pursued graduate study at Cornell University from 1907 to 1909 and at the University of Wisconsin from 1909 to 1910.

From 1904 to 1907 he was instructor and assistant professor in botany at the North Dakota Agricultural College and Experiment Station. From 1907 to 1909 he was assistant in plant breeding at Cornell University. From 1909 to 1910 he was assistant in botany at the University of Wisconsin. From 1910 to the time of his death, Mr. Pritchard was in the employ of the Bureau of Plant Industry, U. S. Department of Agriculture, advancing from agent and assistant physiologist to senior physiologist.

Mr. Pritchard was a persistent and painstaking worker in the field of practical plant breeding and will long be remembered for his development of several varieties of tomato, which were not only resistant to wilt and other diseases but of excellent quality. He was of a kindly disposition and possessed an unusual ability for making friends quickly. He was devoted to his home and family and spent all his spare time there.

LOUIS HERMANN PAMMEL

APRIL 19, 1862-MARCH 23, 1931

Louis Hermann Pammel was graduated from the University of Wisconsin in 1885 with the degree of Bachelor of Agriculture, and in 1889 he received the degree of Master of Science from the same institution. In 1899, he received the degree of Doctor of Philosophy from Washington University (St. Louis), and in 1925 he was honored by the University of Wisconsin with the degree of Doctor of Science.

From 1885 to 1886, Dr. Pammel was private assistant to Dr. W. G. Farlow at Harvard University; from 1886 to 1889, he was assistant in the Shaw School of Botany, Washington University (St. Louis); from 1889 to the time of his death, he was professor of botany at Iowa State College. He did special work for the Texas Agricultural Experiment Station and the Division of Vegetable Pathology, U. S. Department of Agriculture, in 1889; for the Division of Agrostology, U. S. Department of Agriculture, in 1897; for the Bureau of Forestry, U. S. Department of Agriculture, 1899–1902; and for the Bureau of Plant Industry, U. S. Department of Agriculture, in 1902.

He was a charter member of The American Phytopathological Society and a member of a number of botanical societies and academies of science.

Dr. Pammel was a man of unusually broad interests in the entire field of botany. He was a keen observer, untiring collector, ready writer, capable administrator, and inspiring teacher. He was a natural leader, always highly appreciative of the contributions of others. Certainly he strove always "to be of service to humanity."

WARNER JACKSON MORSE

OCTOBER 30, 1872-MARCH 25, 1931

Warner Jackson Morse was graduated from the Johnson (Vermont) Normal School in 1893. From the University of Vermont he received the earned degrees of Bachelor of Science in 1898 and Master of Science in 1903. The same institution bestowed upon him the honorary degree of Doctor of Science in 1923. He pursued his doctorate work at the University of Wisconsin, where he took his degree of Doctor of Philosophy in 1912.

From 1899 to 1901, he was teacher of natural sciences, Montpelier (Vermont) Seminary. At the University of Vermont he was instructor of botany, 1901–1905, and assistant professor in bacteriology, 1905–1906. He was also assistant botanist at the Vermont Agricultural Experiment Station from 1901 until 1906. At the Maine Agricultural Experiment Station he was head of the department of plant pathology from 1906 to June 30, 1923, and director from 1921 to the time of his death on March 25, 1931.

Dr. Morse was a highly capable and productive investigator, a loving husband and father, and a congenial and loyal friend. The known and tried made a much stronger appeal to him than the new and the untested. In keeping with his other sturdy qualities were his unvarying honesty and indiscriminating justness. He was a man of high ideals and at the same time was practical-minded. "To be of service" indeed characterized his every activity, and friendliness and considerateness his every personal relation.

CALVIN HENRY KAUFFMAN

MARCH 1, 1869-JUNE 14, 1931

Calvin Henry Kauffman was graduated from Harvard University in 1895 with the degree of Bachelor of Arts. He spent one year (1901-1902) in graduate study at the University of Wisconsin and two years (1902-1904) at Cornell University. He then continued his studies at the University of Michigan, where he received the degree of Doctor of Philosophy in 1907.

From 1887 to 1890 he taught in the secondary schools of Lebanon, Pennsylvania, and from 1896 to 1898 was principal there. From 1898 to 1900 he taught in a high school at Decatur, Indiana, and the following year in a normal school at Bushnell, Illinois. In 1904 he was appointed to an instructorship in the department of botany of the University of Michigan, where he spent the rest of his life. He became an assistant professor in 1912, associate professor in 1920, and professor in 1931. He also was appointed director of the University Herbarium in 1921. From 1917 to 1919 he was on leave from the University of Michigan, serving as pathological inspector on the Federal Horticultural Board.

Doctor Kauffman was an inspiring teacher and a tireless, enthusiastic investigator. By his thorough and critical studies mycology has been greatly enriched.

VI INTERNATIONAL BOTANICAL CONGRESS

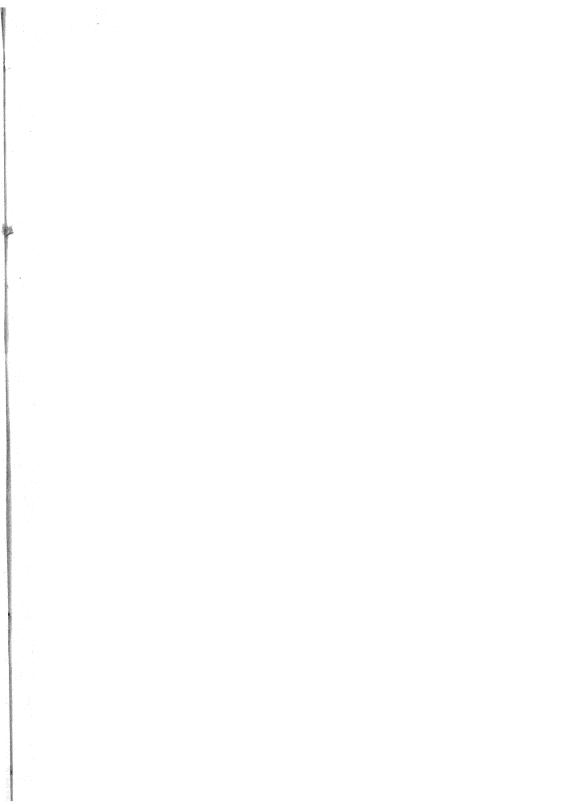
According to a decision by the V International Botanical Congress at Cambridge in 1930, the Sixth Congress will be held in Holland in 1935. An Executive Committee has been formed, President of which is Professor Dr. F. A. F. C. Went (Utrecht), while Professor Dr. J. C. Schoute (Groningen) will act as Vice-President, Dr. W. C. de Leeuw (Bilthoven) as Treasurer and Dr. M. J. Sirks (Wageningen) as Secretary. The Committee has decided that the VI Congress will meet at Amsterdam, September 9th-14th, 1935. Scientific Societies are kindly requested to reckon with these data in planning their own meetings.

VI INTERNATIONALER BOTANISCHER KONGRESS

Laut Beschluss des V. Internationaler Botanischen Kongresses in Cambridge, 1930, wird der Sechste Kongress 1935 in Holland abgehalten werden. Für diesen Kongress hat sich ein Vorbereitungsausschuss gebildet unter der Führung der Herren Prof. Dr. F. A. F. C. Went (Utrecht) Vorsitzender, Prof. Dr. J. C. Schoute (Groningen) Stellvertr. Vorsitzender, Dr. W. C. deLeeuw (Bilthoven) Schatzmeister und Dr. M. J. Sirks (Wageningen) Schrifführer. Der VI Kongress wird vom 9 bis 14 September 1935 in Amsterdam tagen. Wissenschaftliche Gesellschaften wedern freundlichst gebeten, diese Daten bei dr Festellung ihrer Sitzungen berücksichtigen zu wollen.

VI CONGRÉS INTERNATIONAL DE BOTANIQUE

Le V Congrès International de Botanique à Cambridge 1930 a décidé que le Sixième Congrès aura lieu en 1935 en Hollande. Un Comité d'Organisation a été établi sous la direction de MM. le Professeur Dr. F. A. F. C. Went (Utrecht) Président, le Professeur Dr. J. C. Schoute (Groningen) Vice-Président, le Dr. W. C. de Leeuw (Bilthoven) Trésorier et le Dr. M. J. Sirks (Wageningen) Secrétaire. Le VI Congrès se réunira à Amsterdam du 9 au 14 septembre 1935. Les Sociétés scientifiques sont priéss de bien vouloir tenir compte de ces dates en fixant celles d'autres réunions scientifiques.





NATHANIEL ORSON HOWARD

PHYTOPATHOLOGY

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NATHANIEL ORSON HOWARD 1880–1930

WALTER H. SNELL

Dr. Nathaniel O. Howard, Pathologist of the Division of Forest Pathology, United States Department of Agriculture, in charge of the European larch-canker investigations, died suddenly in Providence, R. I., on September 14, 1930. He was in his 51st year.

Dr. Howard was born in Providence, May 11, 1880. He was educated in the city schools and was graduated from Brown University with the degree of Ph.B. in 1903. He received his Sc.M. degree in 1917 and his Ph.D. in 1925 from Brown University. The Rhode Island College of Pharmacy granted him the degree of Pharmaceutical Chemist in 1929. Dr. Howard was a member of Phi Beta Kappa, Sigma Xi, The American Phytopathological Society, American Chemical Society, and Harmony Lodge No. 9, F. & A. M.; he was Secretary and Treasurer of the R. I. Botanical Club, a Fellow of the American Association for the Advancement of Science, and a charter member of Phi Kappa Psi, his college fraternity.

Dr. Howard married Miss Mae Elizabeth Hayes, of North Attleboro, Massachusetts, in 1903. He is survived by his widow, three sons, Edmond Harrison, Walter Henry, and Nathaniel Orson, Jr., and by three brothers, Charles M. Howard, of Pawtucket, R. I., and Harrison C. and Albert W. Howard, of Providence.

Much of Dr. Howard's work in life was teaching. Following graduation Dr. Howard taught science in Coshocton, Ohio, and Rutland, Vermont. In 1905 he returned to Providence, R. I., to teach chemistry at the Technical High School. This he did until 1918. After 1920 he was for several years instructor in the Department of Botany at Brown University, on a part-time basis, teaching morphology occasionally and handling the work of the department in plant physiology and plant chemistry. He also taught a course in advanced plant pathology as a cooperative project between the Department of Botany and the Government Division of Forest Pathology at Brown University. For several years, he also gave the work in botany at the Rhode Island School of Pharmacy.

Dr. Howard entered upon his pathological work first as a part-time collaborator at the Providence office in 1913, investigating the diseases of

shade and ornamental trees and shrubs. In 1918 he was transferred to the office in Forest Products Laboratory at Madison, Wisconsin, to study the control of sap stain, mold, and incipient decay of wood used for wheel spokes and other parts of war vehicles. This work took him through a good part of the hardwood belt of central United States. Shortly after the conclusion of this work, he studied a disease of camphor in Florida. He returned to the Providence office in 1920 to continue work upon shade and ornamental trees until the spring of 1929, when he was placed in charge of the European larch-canker investigations, in which he was engaged until his untimely death.

A loyal and companionable friend, a capable teacher, a cooperative and faithful associate, a devoted husband and father, a scientist of broad interests and ability, Dr. Howard can ill be spared from among us.

Dr. Howard's more important contributions to scientific literature follow:

The control of sap-stain, mold, and incipient decay in green wood with special reference to vehicle stock. U. S. Dept. Agr. Bul. 1037. 1922.

The relation of an undescribed species of Pestalozzia to a disease of Cinnamomum camphora Ness. & Eberm. (Abst.) Phytopath. 13: 47-48. 1923.

Notes on chemical injuries to the eastern white pine (Pinus Strobus L.). Phytopath. 12: 362-368. 1922. (With Walter H. Snell.)

Improved method of sectioning textiles. Textile World. 63: 195-196. 1923. (With Walter H. Snell.)

The relation of moisture contents of wood to its decay. Science, n. s. 62:377-379. 1925. 1923. (With Walter H. Snell and Myron U. Lamb.) A new disease of Douglas fir. Science n. s. 69:651-652. 1929.

Brown University, Providence, R. I.

THE INFLUENCE OF ALTERED HOST METABOLISM UPON MODIFICATION OF THE INFECTION TYPE WITH PUCCINIA GRAMINIS TRITICI p. f. 21

DOROTHY F. FORWARD1

The work of many students of rust parasitism since Marshall Ward has led to the acceptance of the type of infection produced by any given physiologic form of rust upon any given host as an index to physiological compatibility or incompatibility between the host and fungus protoplasts, the nature of which remains unknown. This association has already proved useful in an exploration of the extent of physiologic specialization, especially in the cereal rusts. It should be equally useful in studies of the physiological factors contributing to the state of compatibility or incompatibility, provided a technique for its application could be found. Such a technique is described in the present paper.

There has been a tendency, until very recently, to emphasize unduly the constancy of the infection type within a loosely defined range of experimental conditions, and, indeed, to regard the infection type as a virtually unalterable character proper to a genetical strain of the host in combination with a genetical strain of the rust. But from the standpoint of an inquiry into the nature of the physiological processes concerned in the determination of the infection type, it is not constancy within a certain environmental range but modifiability when the range is overstepped that would be useful as an experimental tool. If, by modifying the processes of the host or of the fungus in a manner definable in terms of the physiology of either, one could at the same time modify the infection type, a program for the investigation of the physiological basis of compatibility or incompatibility would be feasible. The present series of experiments was designed as a first step in what must be an extensive and involved program.

Since the physiology of the rust mycelium cannot, with our present knowledge, be studied apart from the host, the best chances of success obviously lie in attacking, first, the contribution of host physiology to the determination of the infection type. The contribution of the fungus was, therefore, standardized as definitely as possible for these experiments by the selection of a single physiologic form, known to be particularly aggressive and stable in its host relationships. On the other hand, the physiology of hosts infected by this rust form was so modified as to cause modification of infection types. In the present paper, a comparison is made of the effects of several types of experimental treatment on infection type. In

¹ Fellow of the Ontario Research Foundation in the Department of Botany, University of Toronto.

future communications, the modifications of host physiology will be defined in respect to those aspects that the results of this work seem to indicate as significant. Then a comparison of the results of experiments similar to the present ones, but involving numerous rust forms, will be of value in elucidating the nature of the contribution of the pathogen toward success or failure in the establishment of compatible relations between the host and fungus protoplasts.

REVIEW OF LITERATURE

When the experiments reported in this paper were planned, indications already existed that the type of infection produced by one rust form on one host strain is not unalterable, but nothing in the nature of a convincing demonstration of modification of reaction of the host then existed. Since this work was undertaken, however, several such demonstrations have been forthcoming.

In 1905, Ward (29) had reported having cultured Puccinia glumarum (Schm.) Eriks. & Henn. on a susceptible variety of wheat in overheated soil and in a carbon-dioxide-free atmosphere. He was able to reproduce on the susceptible host phenomena similar to those observed on "immune" plants. These phenomena he described as "starvation of hyphae in a nest of dead cells, or the corrosion of cells beneath the spores sown on the leaf"—apparently the same phenomena that, in the system of Stakman and Levine (25), distinguish a resistant from a susceptible infection type. While his lack of knowledge of physiologic specialization of P. glumarum on wheat may leave some doubt as to the purity of his rust material, nevertheless the fact that control plants in a favorable environment showed only susceptible infection types renders it highly probable that his experimental treatment actually caused modification of the reaction of the host plants.

In 1927, Waters (31), in describing corn plants infected with *Puccinia* sorghi Schw, and subjected to darkness for several days wrote as follows:

"In the other plants either the infected leaves died, or the region surrounding the sorus appeared to be completely dried out, leaving the rust mycelium isolated from the food in other regions of the leaf."

It is at least possible that the drying out of tissue around the pustule signified the occurrence of hypersensitive tissue and, therefore, a shifting of the infection type as a result of darkening the host.

Not until 1929, after the present investigation was under way, did further evidence concerning the modifiability of infection types come to my knowledge. In 1929, Gassner and Straib (10) published the results of a number of experiments in which the infection type produced by several cereal rusts on their seedling hosts was altered within limits by culture of the hosts at different concentrations of atmospheric carbon dioxide. Rela-

tive differences between susceptible and resistant varieties were not, however, seriously disturbed. Several publications since 1929 show, on the other hand, that with certain rust-host combinations the infection type is subject to quite radical alteration.

Gassner and Straib (11), Waterhouse (30), Gordon (13), Peturson (24), and Johnson (17) have reported instances where extreme changes in the reaction type of one or more strains of wheat or other cereal to standardized rust forms resulted from changes in the temperature at which the host plants were cultured. Melander (20) reported that uredinia of Puccinia graminis tritici "developed very slowly at 0–1° C. and sometimes the infection type differed from the normal type produced at 10° and 20° C." Waterhouse (30), Gordon (13), and Johnson (17) also mentioned light intensity as a probable factor affecting the infection type in some instances, but no specific investigation of its importance has been recorded. Hassebrauk (16) reported having modified the infection type produced on wheat seedlings by P. triticina by alterations in the mineral nutrients supplied to the seedlings.

Those who have reported changes in infection type appear to have been chiefly interested in their effect on the host relations of the various rust species involved and particularly in their effect upon the identification of physiologic forms. They have not failed to recognize their possible usefulness in a study of the nature of the physiological processes determining the infection type, but almost no experimental work has been done in this direction. Johnson (17) made preliminary efforts to discover differences of structural or chemical constitution between seedlings of the same variety of wheat grown at high and low temperatures but found none. The nature of the relationship of environmental factors to modification of rust infection type, therefore, remains quite unknown.

Environmental factors are known to affect other aspects of rust development, such as the abundance and vigor of infection or the spore form. The consensus of opinion is that where environmental factors affect these aspects of rust development they act chiefly through their effect on host metabolism. This has been demonstrated, at least as regards light, by Mains (19), Gassner (9), and Waters (31). The popular theory of the relation of light to rust development appears to be that the rust is dependent for nutrition upon some substance synthesized by the host in the light. Scant attention has been paid to the possible importance of the catabolic phases of host metabolism in affecting rust development, although Trelease and Trelease (26) have recognized this in relation to the infection of wheat by mildew. Fromme (8) and Mains (19) have described a limited number of experiments in which a period of darkness following inoculation retarded the development of uredinia on susceptible hosts by a period approximately

equal to the duration of darkness. The interpretation placed upon this was that rust development was completely arrested during darkness. The probable cause assigned was the absence of some essential nutritive substance formed by the host only in the light. This conception of the relation of light to rust development appears to have gained popular acceptance. However, very little experimental evidence has been advanced in support of this or, indeed, of any theory of rust nutrition. The true nature of the relation of rust development to host metabolism, like the relation of infection type to host metabolism, remains unknown.

A recent paper by Hanna (15) deals with the relation of host physiology to a general susceptibility to rust. His work constitutes a part of the program of wheat-stem-rust investigation outlined by Newton, Lehmann, and Clarke (21). Hanna's mode of procedure was to measure varietal differences in host vigor in terms of the rates of physiological processes and to search for a correlation of varietal differences in some of the processes with a general susceptibility or resistance to stem rust. He found indications of such a correlation with a high content of carotinoid and chlorophyll pigments but none with diastase or catalase activity or with the rate of respiration over a 2-hour period of leaves of equivalent time age grown in the greenhouse.

MATERIALS AND METHODS

A single physiologic form of the wheat-stem-rust fungus, Puccinia graminis tritici Eriks. & Henn. p. f. 21, has been employed in about 80 experiments in the greenhouse, involving 11 of the 12 standard differential wheat varieties commonly used in the identification of physiologic forms of P. graminis tritici. (Kanred was omitted because it seldom shows any signs of infection by form 21). In pursuance of the policy of trying to produce a definable change in host physiology, the experimental treatment chosen was the confinement of infected and noninfected plants in the dark for more or less prolonged periods of time. Some of the metabolic consequences of prolonged darkness have been determined for certain green plants or plant organs (1-7, 12, 14, 18, 22, 23, 27), and it seemed that a technique such as was used to discover these could be profitably applied to a study of the metabolism of the wheat varieties under experimentation here. Three types of experiment have been used: 1. Continuous-darkness experiments in which seedlings in pots were subjeted to prolonged periods of darkness after rust infection was established. 2. Experiments with shortened daily exposures to light. 3. Continuous-darkness experiments in which detached leaves were subjected to prolonged periods of darkness.

The experiments reported in this paper were all carried out in the greenhouse and extended over a period of about a year and a half. No

environmental control was attempted, with the exception of the use of artificial light, in addition to daylight, for some of the winter experiments.

The differential hosts employed for determination of physiologic forms of *Puccinia graminis tritici* at the Dominion Rust Research Laboratory, Winnipeg, Canada, were grown from seed of known record, obtained from that laboratory.

Only one physiologic form of rust, p. f. 21, has been employed in my experiments, and only this form was introduced during the course of my investigations into the experimental section of the greenhouse that they occupied. Therefore, practically no opportunity for contamination of the form has been present, but occasional inoculation of the complete series of differential hosts was carried out, in order to verify the purity of the form. Form 21 was chosen as a physiologic form particularly aggressive and stable in its host relationships. Seven out of the 12 differential hosts are very susceptible to this rust form, 1 moderately susceptible, and the other 4 highly resistant (Fig. 1). With form 21 none of the differential hosts gives, under natural conditions, the heterogeneous reaction designated by Stakman and Levine (25) as "x."

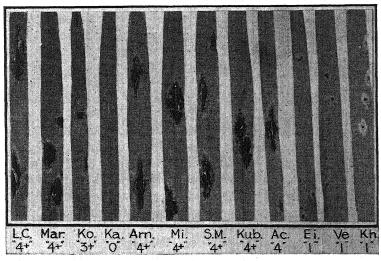


Fig. 1. Reactions of differential hosts to *Puccinia graminis tritici* p.f.21. Photographed 23 days after inoculation. Left to right, 1 leaf each: Little Club "4+", Marquiz "4+", Kota "3+", Kanred (B-2-5) "0," Arnautka "4+", Mindum "4+", Speltz Marz "4+", Kubanka "4+", Acme "4", Einkorn "1", Vernal "1", Khapli "1".

Seedlings were grown in 4-in. pots and were inoculated in the single-leaf stage according to the technique usually employed for the study of stem rust on seedlings. At the time of inoculation a selection was made.

At first, only the evidently atypical seedlings were removed; later, only those were retained that fell within a definite height range. This selection left from 8 to 15 seedlings in a pot. No rigid rule was followed as to the number of plants left in a pot, but observations revealed no differences in experimental results that could be traced to variation in this respect.

In the first three experiments, the development of mycelium in inoculated leaves was followed in free-hand sections, stained with cotton blue in lactophenol. When the mycelium was collecting under the epidermis and the first urediniospores could be seen, the plants were considered ready for subjection to darkness. After the first three trials, it was found that this stage could be recognized from the exterior as the time when small whitish infection flecks on the leaf first become macroscopically visible. Sectioning was accordingly discontinued, and the appearance of infection flecks became the criterion for the stage of development when the plants were ready for transfer to the dark chamber.

At this stage, the endosperm had disappeared from most of the seeds, and many of these had dried up. Certain individuals retained a trace of endosperm, but never more than a trace was observed, although a half-dozen individuals, or usually more, were examined in each experiment.

The dark chamber stood in the greenhouse in which the plants were grown. In the first experiments it was a box 20 in. x 26 in. x 30 in., with ventilation through light baffles, aided by an electric fan in the chamber. Since this chamber would accommodate only about 20 pots, it was replaced by a larger one, 3 ft. x 3 ft. x 4 ft., similarly ventilated. Soon after the large chamber was introduced, use of the fan was discontinued, for it appeared unnecessary.

The temperature of the dark chamber was not controlled in any way. Its daily range of variation was somewhat less than that in the greenhouse. The range varied from about 10° F. in winter to over 30° F. in some weeks of the summer. Temperature changes in both the dark chamber and greenhouse were recorded by thermographs. For the most part, the dark-chamber and greenhouse temperatures fluctuated about approximately the same mean, but that in the dark-chamber failed by 5 to 10° to reach the lower extreme of greenhouse temperature and by 5 to 10° or more to reach the upper extreme. The temperatures of the two environments differed chiefly in range of fluctuations. For this reason and because the temperature differences from one season to another in the greenhouse were as great as or greater than those between greenhouse and dark chamber during any one experiment, it is safe to conclude that the recorded results of confining plants in the dark chamber are attributable to darkness rather than to a temperature change.²

² This has since been definitely proved with plants grown at constant temperature and subjected to periods of darkness at the same temperature.

Changes from greenhouse to dark chamber and vice versa were always made after dark, so that 1 day in the dark means continuous darkness for 2 nights and 1 day, 2 days in the dark means 3 nights and 2 days, and so on.

Except in early experiments, noninoculated control plants and inoculated ones received the same experimental treatment.

Plants, on removal from the dark chamber, were returned to the bench beside untreated controls, both inoculated and noninoculated. Daily notes on their subsequent development were made. These records included information, separately recorded either for each pot or for the two or three pots receiving similar treatment, on the following particulars: The number of leaves bearing open uredinia, the number bearing closed uredinia, those bearing none but with other signs of rust infection, and those showing no signs of infection; the size of the uredinia, their color, and any irregularities of appearance; the estimated proportion of infection centers with uredinia to those without, and the proportion of open to closed uredinia; the presence or absence of chlorotic rings, hypersensitiveness, green islands, or other symptoms of incompatibility; the color and general appearance of the leaves and the amount of dead tissue where any was present. Descriptions of noninoculated plants referred to the color and general appearance of the leaves, and the amount of dead tissue, if any. Plants were kept under observation for at least a month after inoculation, although daily records were not so long continued.

The symbols introduced by Stakman and Levine (25) for the description of types and degrees of infection by physiologic forms of *Puccinia graminis tritici* have been adopted. These symbols were originally intended for use within a certain range of environmental conditions in the identification of physiologic forms of this rust. As convenient shorthand descriptions of the externally visible results of rust infection, however, they may be applied, without violating the definitions set forth by Stakman and Levine, to infection types arising under such a special set of conditions as that arranged for the experiments recorded here. To assist in obtaining a clear conception of my interpretation of these symbols, reference may be made to figure 2.

Since it is important in the present discussion to distinguish between the flecks that always precede pustules and those that represent abortive infections, the former are always referred to as "infection flecks," a term used by Ward (28, p. 276). Abortive infections are distinguished as "necrotic flecks" (Fig. 1, Khapli) or "nonnecrotic flecks" (Fig. 5, B, "4-day" leaves), according to whether a visible area of host tissue is in a collapsed, necrotic state or whether other signs of incompatibility between host and fungus are evident. Nonnecrotic flecks are usually small, whitish areas, definite in outline and quite distinct from the chlorotic areas pro-

duced by rust developing normally on the same host. They resemble an early stage of necrotic flecks, and are definitely hypersensitive although not collapsed and brown. The centers of such flecks may be brown, although no signs of pustule formation are externally visible. Certain small flecks may be entirely brown, but the tissue is not collapsed. These flecks differ distinctly from the ordinary necrotic fleck, but certainly represent abortive infections. The intrinsic relations between the two types of fleck are not understood; the two terms are used simply for descriptive purposes. An attempt is made to be specific as to type whenever flecks are mentioned.

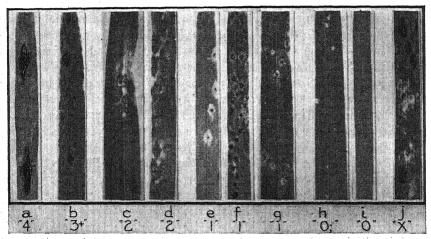


Fig. 2. Rust-infection types. a. "4", Arnautka, Exp. 65, control, photographed 23 days after inoculation. b. "3+", Arnautka, Exp. 64, in dark 1 day, photographed 17 days after inoculation. c. "2", Arnautka, Exp. 31, in dark 2 days, photographed 14 days after inoculation. d. "2", Mindum, Exp. 17b, in dark 2 days, photographed 14 days after inoculation. e. "1", Khapli, Exp. 65, control, photographed 23 days after inoculation. f. "1", Little Club, Exp. 71c, in dark 4 days, photographed 27 days after inoculation. g. "1", Arnautka, Exp. 37, in dark 3 days, photographed 17 days after inoculation. h. "O", Arnautka, Exp. 37, in dark 2 days, photographed 12 days after inoculation. i. "O", (B-2-5) Kanred, Exp. 65, control, photographed 23 days after inoculation. j. "x", Arnautka, Exp. 17a, in dark 5 days, photographed 23 days after inoculation.

EXPERIMENTAL RESULTS:

I. EXPERIMENTS ON SEEDLINGS WITH CONTINUOUS PERIODS OF DARKNESS

A. Conditions of experimentation. The majority of experiments have been in the nature of subjection of plants to continuous periods of darkness. All the plants of one sowing (except those in the control pots) were placed in the dark chamber at the same time, and lots of 2, or sometimes 3, pots of inoculated plants and the same number of noninoculated ones were removed daily until all had been returned to the light. For example:

	No. of pots placed in the	No. of pots removed after continuous darkness for:								
	dark at one time	1 day	2 days days		4 days	5 days	6 days	7 days		
Inoculated	14	2	2	2	2	2	2	2		
Noninoculated	14	2	2	2	2	2	2	2		

The maximum sojourn in the dark varied from 3 to 7 days in the many experiments performed.

About 70 experiments of this type were conducted in the greenhouse between March, 1929, and June, 1930, involving 11 of the differential hosts.

B. Effect of continuous darkness upon the appearance of host tissue. Leaves of all the varieties of wheat in the dark chamber, whether infected³

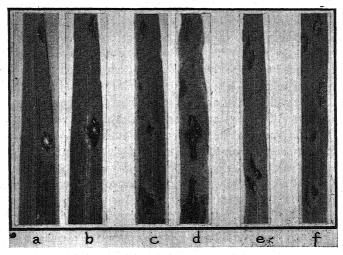


Fig. 3. a-e. Arnautka, f. Little Club. Secondary development. a. Arnautka, Exp. 34, inoculated Sept. 12, 1929, placed in dark Sept. 18, photographed Sept. 27. In dark 2 days. b. The same leaf as a, photographed Oct. 1 (4 days later). c. Arnautka, Exp. 64, inoculated Apr. 4, 1930, placed in dark Apr. 11, photographed Apr. 21. In dark 1 day. d. The same leaf as c, photographed May 2, 1930 (11 days later). e. Arnautka, Exp. 34, inoculated Sept. 12, 1929, placed in dark Sept. 18, photographed Oct. 1. In dark 4 days. f. Little Club, Exp. 66, inoculated Apr. 10, 1930, placed in dark Apr. 16, photographed Apr. 29. In dark 6 days.

or free from rust, underwent a series of changes in appearance, beginning with a slight paling that gradually increased in severity until it reached complete yellowing. This was sometimes followed by a bleaching from yellow to white. In the later stages of etiolation the leaves lost their turgor

3 This general statement excludes the tissues in the immediate vicinity of infection centers. These come under discussion in the section on infection types.

and often took on a translucent, or injected, appearance. This was accompanied by browning and drying of the tissues. All parts of a single leaf did not progress at the same rate through this series of changes. There was a gradient from more rapid progression at the tip of the leaf to slower change at the base. Thus it happened that the first visible effect of darkness on any leaf was a slight paling of the tip. With prolonged darkness it became more chlorotic and the slight paleness progressed down the leaf. In the same way the first dead tissue appeared at the tip of the leaf and progressed downward with longer duration of darkness. The effect can be seen in figures 6, C, and 10, A,4 where the number under each leaf represents the number of days in continuous darkness. Leaves infected by rust behaved essentially as did the noninfected ones (Figs. 5, C, 7, C, 8, A, and others).

These photographs were all taken some days after removal of the plants from the dark chamber, and the appearance of the leaves was, therefore, determined by the period of light following darkness as well as by the period of darkness itself. Those portions of the leaves that were dead when photographed were not always dead on removal from the dark. The tissues appeared to reach a point in the series of changes resulting from darkness beyond which these changes were irreversible, and they proceeded to the point of death even after return to natural light conditions. On the other hand, leaf tissue that had not reached such a point of irreversibility in the dark chamber was able to survive when placed in a favorable environment. For purposes of the present paper this crude distinction between reversibility and irreversibility of the effects of prolonged darkness will suffice.

By a comparison of the various illustrations it is evident that the rate of progression through the series of changes resulting from darkness was not the same in all experiments. The rate seemed to depend upon the wheat variety concerned, the environmental conditions during the sojourn of the plants in the dark, chiefly a matter of temperature, and the physiological state of the plants when placed in the dark, chiefly a function of age and of environmental conditions during growth. It will be observed also that in the experiment represented by figure 6, C, and others, leaves receiving the same treatment simultaneously were not always alike in their response. This sort of individual variation in behavior was frequently noted and was associated with similar heterogeneity in rust development. Certain experiments designed to discover the underlying cause for this variation will be mentioned later. A more detailed account of the dependence

⁴ In figure 10, A, the white flecking at the tip of the leaf held 1 day in the dark is not a result of this treatment, for similar flecking occurred on many of the untreated leaves in this particular experiment.

of rapidity of change in appearance on environment and on the physiological state of the tissues will appear in later sections together with a discussion of its significance in relation to rust development.

Such a series of changes in appearance as has been described for wheat seedlings confined in the dark must be considered as the outwardly visible signs of a series of metabolic changes resident in the cell protoplasm. Loss of green color is consequent upon the breakdown of chlorophyll; loss of turgor upon a permeability change. Such fundamental changes could not occur as isolated processes in the complex of reactions comprising the metabolic activity of green plant tissues in the dark. The host tissues confined in the dark must, therefore, be thought of as undergoing a series of metabolic changes to the time relations of which the outwardly visible changes provide an index.

In cherry-laurel leaves changes in appearance resulting from darkness have been definitely correlated with more accurate indices of changing metabolism. According to Godwin and Bishop (12), F. F. Blackman found that yellowing of detached mature cherry-laurel leaves in the dark was associated with an increase and subsequent decrease in respiratory activity following an initial decline. Godwin and Bishop, themselves, reported that when in young leaves of the cherry laurel an increase in respiration failed to appear after the initial decline yellowing also failed, the green color giving way directly to browning and general disintegration after prolonged subjection to darkness. The same authors found a very intimate correlation between rate of yellowing, rate of loss of cyanogenetic glucosides, and respiratory intensity in cherry-laurel leaves subjected to darkness.

There is every reason to believe that in wheat leaves, also, the rate of change in appearance can be associated with the rate of progression through the sequence of physiological states characteristic of the metabolism of the tissues in continuous darkness. Whether the correlations are the same as in cherry-laurel leaves is a matter for experimental determination. Present indications from experiments in progress are that, in this respect, wheat leaves present certain dissimilarities from those of cherry laurel.

C. Effect of continuous darkness upon the rate of rust development. The results recorded in this section are concordant with those of other workers inasmuch as subjection of infected plants to darkness during the "incubation period" of the fungus increased the time required for the development of pustules. On the other hand, the indication that others who have studied the problem have discovered that the incubation period is lengthened by a number of days equivalent to the number of days spent in the dark has not been borne out by my investigations. The actual num-

⁵ In accordance with previous usage in rust literature, the term includes the period from inoculation to the appearance of pustules.

ber of days added to the incubation period by a definite sojourn of the host plants in darkness varied considerably under different circumstances, as will appear in the following account of experimental results.

It is not easy to express accurately the effect of darkness on the rate of rust development. Fromme (8), Mains (19), and Waters (31) recorded this effect in terms of number of days of retardation in the development of uredinia, and a similar system has been adopted here. In my experiments, however, the experimental treatment caused not only retardation but also inhibition of pustule development. Pustules did not always form in infection flecks and, when they did form, did not always open, nor did they always reach their full size. Lengthening of the incubation period was but one of the effects of darkness.

The length of the period of time from the first appearance of infection flecks to the point where most of these bore pustules as brownish swellings under the epidermis was selected as an index to the rate of development of the rust. The compilation of daily notes upon the appearance of infected leaves made possible a comparison of the progress of the rust at various periods of development, and it turned out that prolongation of the incubation period was accomplished chiefly in the period selected. earlier part of the incubation period was not affected, for it was only at its close, when infection flecks first appeared, that the plants were placed in the dark chamber. The interval during which pustules were breaking through the epidermis was sometimes affected by a period of darkness, but it did not necessarily increase in duration. On the contrary, it was sometimes curtailed after considerable periods in the dark. The explanation of this is that when pustules were formed on plants left long in the dark they often opened while still very much smaller than nonerumpent pustules on susceptible control plants.

The duration of this intermediate period of rust development having been selected as the best single numerical index of the rate of development of the rust, the information derived from all of my experiments concerning the effect of continuous darkness upon this period is presented in table 1.

The number of days between the appearance of infection flecks and the development of pustules in most of these, under different treatments, was determined by a scrutiny of my daily greenhouse records, showing the proportion of open to closed pustules and of infection flecks with pustules to those without. The proportion was estimated after each leaf had been examined carefully and the number of leaves bearing open pustules, those bearing closed pustules, and the number bearing infection flecks without pustules were taken into account. If, for any reason, it was impossible to tell with reasonable certainty from my records on which of two days the

TABLE 1.—A summary of experimental results concerning the effect of continuous darkness upon the rate of pustule development of Pucchita graminis tritics v. f. 21 on 11 differential host varieties

Lapse of time in days between the first appearance of infection flecks and the stage when most of these had formed pustules, after subjection to darkness for:	5 days 6 days 7 days	$ \begin{array}{c ccccccccccccccccccccccccccccccccccc$	
ppearance of in ules, after sub	4 days		1 1
n the first al	3 days	VIIII 7-8 (9)° (7-8) (7-8) (13-15) 5 6 (11-12) 6 6 6 6 6 6 6 6 6 6 6 6 6 6 6 6 6 6 6	9 8-7 8-9 8-9 1
lays betweed these had f	2 days	VIII 7-6 6-7 6-7 9-10 7-8 7-8 7-8 7-8 8-7 7-7 7-8 8-7 8-7	8 6-7 5-6 6-7
time in c most of	1 day	VI VI C C C C C C C C C C C C C C C C C	4 6 4 4 4 4 5 4 4 4 4 4 4 4 4 4 4 4 4 4
Lapse of when	0 day	> ² 2 2 2 2 2 2 1 1 2 2 2 4 4 2 2 2 2 2 2 2	0101 0101
Time from inoc. to appearance of	infection flecks Days	$\sum_{\mathbf{q}} \mathbf{r} \sim \mathbf{r} $	987991
Date of	inoculation ,	HI (1. 29, 6.6 (2. 20, 6.6 (3. 22, 6.6 (4. 22, 6.6 (4. 24, 6.6 (5. 20, 6.6 (6. 20, 6.6 (7. 20, 6.6 (8. 23, 6.6 (8. 23, 6.6 (9. 23, 6.6 (9. 23, 6.6 (1. 20, 6.6 (1. 20, 6.6 (2. 20, 6.6 (3. 20, 6.6 (4. 20, 6.6 (5. 20, 6.6 (6. 20, 6.6 (7. 20, 6.6 (7. 20, 6.6 (8. 20, 6.6 (9. 20, 6.6 (1. 20, 6.6 (Mar. 15, 29, 4, 28, 4, Apr. 10, 4, May 29, 4, 4, 29, 4, 4, 29, 4, 4, 4, 4, 4, 4, 4, 4, 4, 4, 4, 4, 4,
Exp.	no.	H 10a 112a 114c 115c 22 24 24 30 43 49 49 49 50a 56b 56b 65 66 66	6 7 10b 21a 21b
Vonicety	variety	Ir Little Club	Marquis

TABLE 1.—Continued

he stage for:	7 days	XII						1					
flecks and ti darkness	6 days	XI	1	i 1 1		1		1	1 1			(7-8) or –	1
f infection subjection t	5 days	×	1	1 [1	ı	1.1	(12-13)	1	l I		(10)	(1)	I .
ppearance o ules, after s	4 days	XI	$\begin{pmatrix} 11-13 \\ 8-9 \\ - \end{pmatrix}$	(9–10)	(11)	(TT) 1	(11-12)	1	1 1		I I	(9-10)	1
Lapse of time in days between the first appearance of infection flecks and the stage when most of these had formed pustules, after subjection to darkness for:	3 days	VIII	9–10 8–9	10-11	o (§	(12)	(6-8)	(7–8)	1 1	5 or more	4-5	∞ r⊃ 1	1
lays betwee these had	2 days	ПУ	2-8 6-8 7	6-8	- 1	- 7 - 4	7-8	2-9	စ	2-9	(9)	7 5 6–7	(2)
time in d most of	1 day	ΔI	6 5–6 4	5-6 4		34	5-6	4		4	es 4	444	5
Lapse of when	0 day	Δ	4-5	2 2 2 2	ာ ေ	N 61 C	1 [€] °	ാനെയ	10101	C 3	1 6	3 1-2 2-3	ro 03
Time from inoc. to appearance of	infection flecks Days	IV	70 0 4	H & & C	٥ (o 10 a	2-9			1	9	2 9 2	7 9
Date of	inoculation	III	Apr. 20, '29 May 16, ''	20,7,	4,			, 20° 20°, 40°, 40°, 40°, 40°, 40°, 40°, 40°, 4	344	Apr. 10, '29	May 8, "" "" 11, "" "" 11, 30	May 3, 29 Apr. 27, 30 May 11, "	May 8, "
Exp.	no.	П	12b 19	8688	£ ,	17b	34 r	37	64a 64b	10e	16b 17a 77	15b 73 76	75
Varioty		J.I	Kota			Arnautka				Mindum		Speltz Marz	Kubanka

TABLE 1.—Continued

he stage for:	7 days	XII		1	_										-	-				
lecks and to darkness	6 days	XI					1			1		1							!	
infection fubjection to	5 days	×	ī	1	ľ		1			1		1			l		ı			
Lapse of time in days between the first appearance of infection flecks and the stage when most of these had formed pustules, after subjection to darkness for:	4 days	IX	l	ı	ı	1	1			l		1		(14)		1	1			
ı the first ay ormed pustı	3 days	VIII	(>15)	1	6		(2-2)	1	1	1		1		(12-13)	-	1	1		1	-
ays betweer these had f	2 days	VII	4-5	2-4	4-5				(11-12)	8 or	more	(11)	<u> </u>	(11)		(13)	<u></u>			1
time in d most of	1 day	IA	3-4	1-2	4-5	3-4	က		(6)	(8-9)		(8-9)		∞		10	(6<)		-	
Lapse of when	0 day	Δ	1-2	-	c 3	က	1-3	67 -3	6)	8 or	more	3-6)	∞	2-9	°C	. 67 . 69	4	ភ	
Time from inoc. to appearance of	infection flecks Days	IV	7		5	rc	9	9		7		о к	>	2	9	ç	, <u>L</u> -	∞	7	
Date of	inoculation	H		2		-	Π,			Sept. 30, "		Apr. 7, 30		May 3, '29	Apr. 7, '30	06				
Exp.	no.	L	169	23	98	32	40	65	14b	38	-	38 5	4	15a	65	10,	900	35.	20	65
	Variety	JI	Agmo	Acme					Rinkorn					Vernal		1	Knapu			

a Continuous artificial light was added to daylight.

b A series of dots (......) signifies that the data required to fill a certain space were lacking.

c Brackets around a number signify that all of the infection flecks did not produce pustules.

d A minus sign (-) signifies that few or none of the infection flecks developed pustules as long as the plants were kept under observation or until they died.

• One pot required 3 days, 1 pot required 6 days.

1 The Roman numerals at the heads of columns are introduced merely for convenience in referring to the contents of the table.

end of a development period had been reached, the possible alternatives for the duration of the period were recorded (e.g., 2-3, 4-5). For a few experiments, open pustules, closed pustules, and flecks without pustules were actually counted.

Table 1, column IV gives the duration of the first period in rust development, the time from inoculation until the first appearance of infection flecks. This column is included to indicate the rate of development of the fungus previous to subjection to darkness in the various experiments. The addition of the values in this column to the values for corresponding experiments in columns V-XII will give values that represent the incubation periods after the various treatments, for they represent the time from inoculation until most of the pustules have become visible and a few of them have ruptured the epidermis. Columns V-XII deal directly with the duration of the period of pustule formation. Column V shows the number of days required on control plants allowed to develop undisturbed in the greenhouse. Columns VI-XII show the time required for pustule formation after subjection of the plants to darkness for 1 to 7 days, beginning with the inception of the period of pustule development.

It is obvious from a study of this table that the treatment caused a prolongation of this period but that the prolongation was not always equivalent to the period of darkness. For instance, 1 day in the dark might retard pustule development either not at all or by 1 day or by more than 1 day; even a 6-day retardation was once recorded for Khapli. The general tendency was for pustule development to be retarded a longer time than that spent in the dark by the infected host plants.

The possible confusion of secondary with delayed primary development (p. 522) presents a source of error in recording retardation in rust development. If, however, this error affects the records, it masks rather than accentuates the discrepancy between the duration of darkness and the retardation in primary rust development. This discrepancy may, therefore, be even greater than it appears to be in these records.

Recapitulation. In every experiment darkness delayed pustule formation, the period of delay being roughly proportional but not necessarily equivalent to the time in darkness. The numerical index chosen was regarded as the best medium available for expression of the effect of my experimental treatment on the rate of rust development. If duration of the incubation periods had been presented, it would have shown practically the same discrepancy between the dark period and prolongation of the incubation period. It is not intended that precise significance should be attached to these numerical values. The fact that secondary development could not always be distinguished from primary made difficult precise measurement of rate of development, as did also the fact that primary rust development was

often not merely retarded but modified and inhibited (p. 510). Rates of development in different experiments are not comparable because of insufficient standardization in the age of the seedlings employed and because of differences in environmental conditions. Rates of development in any one experiment, however, are comparable, and, in spite of the variety of experimental conditions introduced, in no case were these so adjusted as to cause equivalence between retardation and duration of darkness in all of the periods of darkness involved in one experiment. Isolated instances of equivalence occurred, but so infrequently as to make them seem of no general significance.

These observations differ from those of Fromme (8) and Mains (19), who, in a few experiments with Puccinia coronata on oat seedlings, discovered indications that subjection to darkness after inoculation caused a period of retardation in pustule development equal to the period of darkness. Obviously, such indications do not find substantiation under the conditions of my more numerous experiments with P. graminis tritici on wheat seedlings; nor does the theory based upon the equivalence of the two periods in question, namely, that during the time the host is in the dark the development of the fungus is arrested because of cessation of carbon assimilation in the host tissues, and on return to light, proceeds at its previous pace. Mains (19) cited one instance where retardation in rust development exceeded the 11 days of darkness. This he treated as an exceptional case, suggesting that the extremely long dark period had disarranged the physiological processes of the host so that on its return to light these were carried on poorly and were, therefore, unable to furnish the fungus enough food to allow it to resume growth immediately at its previous rate. A discrepancy between retardation and the duration of darkness, treated as exceptional by Mains, proved very general in my experiments. His explanation of the single case in which retardation exceeded the dark period does not adequately account for the results of my experiments. As already pointed out (p. 503), host physiology is regarded as entering upon an altered course as soon as the plants are transferred to darkness, and, whether the dark period be long or short, the physiologic state of the tissues, not only during the light period following darkness but also during the dark period itself, is considered important in determining the degree of retardation in rust development. Furthermore, there is evidence that rust development in my experiments was not completely arrested during darkness. Minute pustules have been seen to develop and break open while the host was in the dark chamber. The following pages will bring additional evidence that during the sojourn of the host in darkness development of the rust was not simply arrested but its relation to the host was strongly modified and its development retarded, presumably because of the modified host relations resulting from darkness.

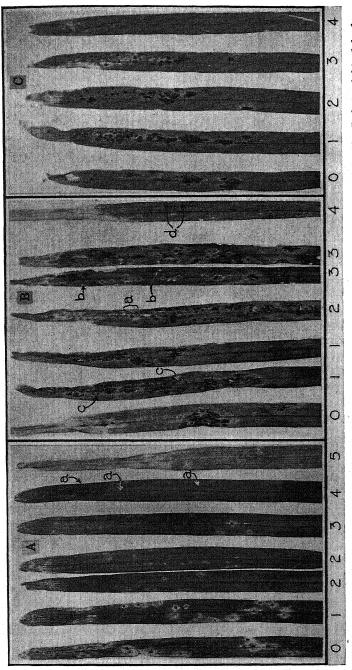
D. Effect of continuous darkness on infection type. Figure 2, representing infection types of wheat stem rust as defined by Stakman and Levine (25), represents some standard reactions of wheat varieties to rust forms and is a composite photograph partly of control leaves and partly of leaves held in darkness for different periods of time. It includes 5 varieties of wheat infected by Puccinia graminis tritici p. f. 21. (See explanation of plate.) The ordinary reactions of these 5 varieties to form 21 are presented in figure 1. It will be observed that the one variety, Arnautka. infected with the single rust form, appears in figure 2 as a representative of every type of infection. Sufficient continuous sojourn of infected wheat seedlings in the dark at the time when pustule development is in progress has evidently resulted in a modification of those symptoms that indicate the degree of compatibility between host and pathogen and are the basis for expressing the standard reactions of the differential hosts to physiologic forms of P. graminis tritici. About 70 experiments with seedlings subjected to periods of continuous darkness have had this result in common.

Figures 4–9 illustrate the outcome of several experiments. Photographic records of nearly all the experiments were obtained, but it is, of course, impossible to present them all here. Those have been selected that illustrate best the nature of my experimental results. Each photograph includes leaves that represent the results of one experiment. In figure 4, A, for example, the first leaf on the left was selected as representative of plants allowed to develop naturally; the next leaf was one of those deprived of light for 1 day; and the rest, those deprived of light 2, 3, 4, and 5 days. The progression is always from untreated leaves, at the left, to those longest in darkness, at the right. For convenience in relating a photograph to its proper experimental treatment, a number is printed below each leaf to indicate the number of days of darkness to which the leaf was subjected.

One leaf in the photographs represents 2, or sometimes 3, pots, *i.e.*, 15 to 40 1-leaf plants. It was often difficult to choose a single leaf entirely representative of all the plants in the lot, for there was, as with effects on the general appearance of the host tissues, a certain amount of individual variation amongst the plants in regard to the effect of any period of darkness on the infection type. Where the variation was too great, more than one leaf was included in the illustration.

In one of the photographs (Fig. 8, A) a noninoculated leaf is to be seen beside each rusted leaf. Photographs of noninoculated controls were made for many of the experiments, but for the most part separately from those of inoculated leaves. Most of these are omitted, and reference is made to non-inoculated controls by description only.

Most of the photographs were taken when the rust on control plants had reached full development, that is, about 15 to 20 days after inoculation.



C. Speltz Marz, Exp. 15b, inoculated May 3, 1929, placed in dark May 10, photographed May 21. Left to right; 1 leaf treated, 1 leaf in dark 1 day, 2 leaves in dark 2 days, 1 leaf each in dark 3 days, 4 days, 5 days. a. Minute pustules on a leaf in treated, 2 leaves in dark 1 day, 1 leaf in dark 2 days, 2 leaves in dark 3 days, 1 leaf in dark 4 days. a. Minute pustules in green b. Minute necrotic flecks without pustules. c. Minute pustules on leaves in dark 1 day. d. Green areas marking infection Fra. 4. A. Khapli, Exp. 22, inoculated June 3, 1929, placed in dark June 10, photographed June 20. Left to right: 1 leaf undark 4 days. B. Acme, Exp. 16a, inoculated May 8, 1929, placed in dark May 15, photographed May 30. Left to right: 1 leaf uneach: untreated, in dark 1 day, 2 days, 3 days, 4 days. islands. centers.

This was considered the best time to illustrate the effect of darkness by a single photograph, although no single photograph can be entirely adequate because of the progressive nature of rust development under the influence of the period of darkness imposed and the period of natural illumination following it (p. 524).

A complete set of experiments on 11 differential hosts was carried out during the spring and early summer of 1929, and several of the varieties were used in the same season of 1930. One experiment on each variety is described in this section.

KHAPLI, EXPERIMENT 22 (Fig. 4, A)—24 pots planted May 27, 1929. 12 pots inoculated June 3. To dark chamber June 10. Photographed June 20, 17 days after inoculation, 4 days after return of "5-day" plants to light. (The "4-day" leaves had, therefore, been in the light 5 days after their sojourn in the dark, the "3-day" leaves 6 days, etc.6

Reaction of controls-"1".

Effect on infection type of continuous darkness for: One day—some flecks without pustules. Two days—reaction "1=", i.e., flecks small, very few with pustules. Three, four days—further decrease in size of flecks and number of pustules. (The pustules on the 4-day leaves photographed are so minute as to be difficult of discovery but are present in Fig. 4, A). Five days—no pustules, no necrosis, indefinite nonnecrotic flecks with green rings, as in Fig. 4, A.

ACME, EXPERIMENT 16a (Fig. 4, B)—12 pots planted April 26, 1929. Inoculated May 8. To dark chamber May 15. Photographed May 30, 22 days after inoculation, 11 days after return of the 4-day leaves to light.

Reaction of controls-"4c".

Effect on infection type of continuous darkness for: One day—small pustules with large ones. (At the time of photographing, 22 days after inoculation, the small pustules were pretty well hidden, but a few can be picked out at 4, B, c. Such small pustules may occur at times in "4" reactions, but in this experiment the 1-day plants could be distinguished from the control plants by the presence of these minute pustules).

Two days—"x' reaction, i.e., greater proportion of small pustules, some in necrotic, some in nonnecrotic flecks. (It will be observed that in the leaf photographed most

⁶ The length of time the leaves shown in any of the figures had already been exposed to normal light conditions after removal from the dark chamber can always be computed from the information given in the legends accompanying the figures. The reader may be assured that, although it was impossible to avoid a discrepancy with regard to the times the various leaves in a figure had been allowed to recover, the effects of this discrepancy were small as compared with the actual effects of the different periods of darkness.

7"Green rings" are distinguished from "green islands." The latter term implies, in this paper, a hypersensitive halo (Fig. 2 e, d); the former, a ring of tissue around a rust infection that remained a deeper green than the surrounding tissue after subjection to darkness (Fig. 8, C, base of 5-day leaf). Such rings always appeared on plants left in the dark long enough to cause paling. Green rings may be narrow or broad and grade into "green areas."

of the large pustules present are toward the base of the infected area. Toward the upper end at 4, B, a, are a few pustules in small green islands, surrounded by necrotic halos. It was frequently observed with other varieties, as well as with Acme, that, especially in leaves held for shorter times in the dark, the more "susceptible" types of pustules predominated toward the base of a leaf, while the more "resistant" types predominated toward the tip (Figs. 4, B and C, 7, A). This distribution of types is associated with the physiological gradient already mentioned (p. 502) and at all times apparent in the leaves of noninoculated as well as inoculated seedlings kept Three days-"x" reaction, i.e., a few large pustules intermingled with resistant types strikingly like Khapli, although necrosis not so extensive as that usually resulting from infection of Khapli; a few minute necrotic flecks, as at b, developed very early. (The large pustules accomplished their entire development during the 11 days that elapsed between returning the leaves to natural light conditions and photographing them.) Four days—only 1 pustule at any time; this appeared 8 days after return to light and 10 days later had reached normal size; all other infections minute; indistinct, nonnecrotic flecks in dark green areas on paler leaf, e.g., at d in figure 4, B; only two leaves survived until photographed. Five days-survived only 3 days after return to light, no pustules, green areas on pale leaves, as 4-day leaves.

VERNAL, EXPERIMENT 15a (Fig. 5, A)—9 pots planted April 22, 1929. Inoculated May 3. To dark chamber May 8. Photographed May 21, 18 days after inoculation and 9 days after return of 4-day leaves to light.

Reaction of controls-"1".

Effect on infection type of continuous darkness for: One day—little change except in rate of development. Two days—smaller pustules. Three days—no pustules until 14 days after inoculation (6 days after return to light), then fairly numerous pustules, as in figure 1. Four days—one pustule on 7th day after return to light, then fairly numerous pustules.

Speltz Marz, Experiment 15b (Fig. 4, C)—9 pots planted April 22, 1929. Inoculated May 3. To dark chamber May 10. Photographed May 21, 18 days after inoculation and 7 days after return of 4-day leaves to light.

Reaction of controls-"4".

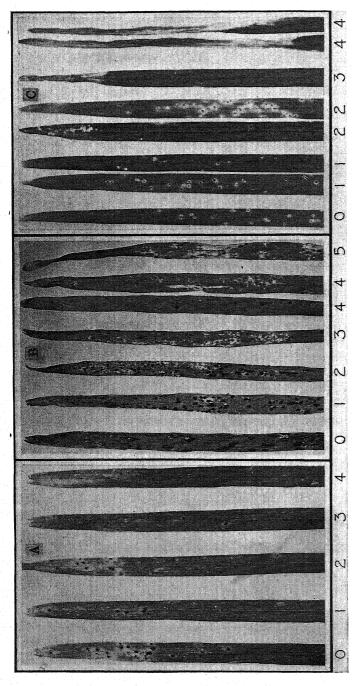
Effect on infection type of continuous darkness for: One day—no change. Two days—almost no change, development slower. Three days—''x' reaction. Four days—very few large pustules, practically all small with nonnecrotic flecks, a few green islands.

MINDUM, EXPERIMENT 17a (Fig. 5, B)—12 pots planted April 30, 1929. Inoculated May 11 (4 in. high or more). To dark chamber May 18. Photographed May 29, 18 days after inoculation and 6 days after return of the 5-day leaves to light.

Reaction of controls-"4" (not quite completely developed when photographed).

Effect on infection type of continuous darkness for: One day—a little hypersensitive tissue, most pustules soon developed to "4" type. Two, three, four, five days—

⁸ The term "hypersensitive tissue" is used in a descriptive sense to indicate the visible result of a definite incompatibility between host and pathogen. It includes necrotic tissue and such nonnecrotic tissue as is sufficiently well defined to be distinct from the more diffuse "chlorotic tissue."



graphed May 29. Left to right, 1 leaf each: untreated, in dark 1 day, 2 days, 3 days; 2 leaves in dark 4 days; 1 leaf in dark 5 Fig. 5. A. Vernal, Exp. 15a, inoculated May 3, 1929, placed in dark May 8, photographed May 21. Left to right, 1 leaf each: days. C. Binkorn, Exp. 72, inoculated Apr. 26, 1930, placed in dark May 1, photographed May 7. Left to right: 1 leaf untreated, Untreated, in dark 1 day, 2 days, 3 days, 4 days. B. Mindum, Exp. 17a, inoculated May 11, 1929, placed in dark May 18, photo-2 leaves in dark 1 day, 2 leaves in dark 2 days, 1 leaf in dark 3 days, 2 leaves in dark 4 days.

greater proportion of hypersensitive tissue. Response similar to Arnautka, experiment 17b.

Kubanka, Experiment 14a (no illustration)—9 pots planted April 13, 1929. Inoculated April 23. To dark chamber April 28.

Reaction of controls-"3" reaction as a result of very heavy infection.

- Effect on infection type of continuous darkness for: One, two days—a little hypersensitive tissue. Three days—''x''—(variation from minute pustules in nonnecrotic flecks to normal pustules); certain areas almost entirely etiolated, with very minute pustules. Four days—resembled 3-day leaves, but the symptoms of incompatibility recorded as more pronounced.
- ARNAUTKA, EXPERIMENT 17b (Fig. 7, A)—12 pots planted April 30, 1929. Inoculated May 11 (4 in. high or more). To dark chamber May 17. Photographed June 3, 23 days after inoculation and 12 days after return of 5-day plants to light.

Reaction of controls-"4".

- Effect on infection type of continuous darkness for: One day—no change in reaction, development slower. Two days—tiny pustules in nonnecrotic flecks among large ones. Three, four, five days—proportion of "resistant" to "susceptible" pustules increased. As in the other varieties, the susceptible pustules were always later in developing than the resistant ones.
- EINKORN, EXPERIMENT 72 (Fig. 5, C)—21 pots planted April 16, 1930. Fourteen pots inoculated April 26 (3-3\frac{3}{4} in. high). To dark chamber May 1. Photographed May 7, 11 days after inoculation, 2 days after return of the 4-day leaves to light.

Reaction of controls-"1",

- Effect on infection of continuous darkness for: One day—unchanged except in rate of development. Two days—more diffuse hypersensitive areas (as shown in one 2-day leaf in figure 5, C). In the center of the indefinite yellow area a sharply defined translucent area usually appeared. In this area a pustule sometimes developed. Three days—flecks, for the most part minute, necrotic or white and nonnecrotic, or merely green specks on a paler background visible only by transmitted light. Two days after photograph for figure 5, C, was taken and 5 days after the period of darkness was completed, the 3-day leaves produced 2 minute pustules. Still later, pustules appeared in several flecks but never in so many as ½ of them, whereas on control leaves, pustules appeared in § of the flecks. Four days—no pustules at any time, very few flecks. Many leaves died within 2 or 3 days after their return to light. Five, six days—dead on removal from the dark chamber.
- MARQUIS, EXPERIMENT 21a (Fig. 6, B)—20 pots planted May 21, 1929. Ten pots inoculated May 29 (2-3 in. high). To dark chamber June 4. Photographed June 11, 13 days after inoculation and 2 days after return of the 5-day leaves to light.

Reaction of controls—"4-c" (not fully developed when photographed).

Effect on infection type of continuous darkness for: Two days—when photographed tiny pustules and definitely chlorotic areas, later developed type-"3" infection. Three days

⁹ The terms "resistant" and "susceptible" are used in a descriptive sense to indicate the sort of infection centers that go to make up a resistant or susceptible infection type as defined by Stakman and Levine (25).

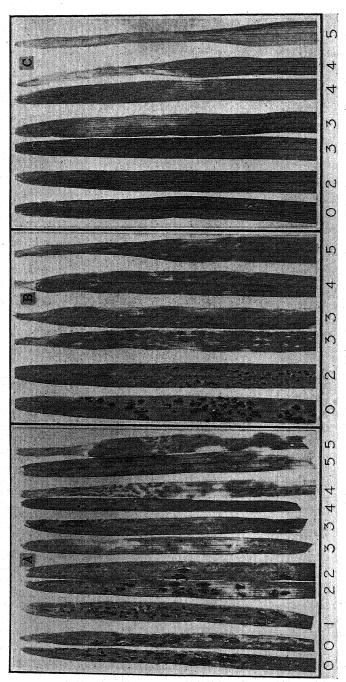


Fig. 6. A. Little Club, Exp. 20, inoculated May 22, 1929, placed in dark May 28, photographed June 10. Left to right: 2 leaves untreated, I leaf in dark 1 day, 2 leaves in dark 2 days, 2 leaves in dark 3 days, 2 leaves in dark 4 days, 2 leaves in dark 5 days. B. Marquis, Exp. 21a, inoculated May 29, 1929, placed in dark June 4, photographed June 11. Left to right: 1 leaf untreated, 1 leaf in dark 2 days, 2 leaves in dark 3 days, 1 leaf in dark 4 days, 1 leaf in dark 5 days. C. Marquis, Exp. 21a, noninoeulated, placed in dark June 4, photographed June 11. Left to right: 1 leaf untreated, 1 leaf in dark 2 days, 2 leaves in dark 3 days, 2 leaves in dark 4 days, 1 leaf in dark 5 days.

—green islands, particularly striking on some leaves. Minute pustules had developed in some of the green islands when the photograph was taken. Three days later these were as large as "3" pustules, but a certain amount of hypersensitive tissue remained visible. Four days—green islands, further suppression of pustules. Five days—no pustules, indefinite nonnecrotic flecks with green islands or rings.

KOTA, EXPERIMENT 19 (Fig. 8, A)—20 pots planted May 6, 1929. Ten pots inoculated May 16. To dark chamber May 22. Photographed June 1, 16 days after inoculation and 6 days after return to the 4-day leaves to light.

Reaction of controls-"3++c" or "4=c".

Effect on infection type of continuous darkness for: One day—no change in reaction. Two days—green islands with necrotic rings that, in areas of crowded infection, appeared as irregular necrotic patches, sometimes forming a network. Three days—more necrosis, smaller pustules. Four days—about as the 3-day leaves.

LITTLE CLUB, EXPERIMENT 20 (Fig. 6, A)—20 pots planted May 14, 1929. Twelve pots inoculated May 22 (2-3 in. high). To dark chamber May 28. Photographed June 10, 19 days after inoculation and 7 days after return of the 6-day leaves to light.

Reaction of controls—"4++". (White speckling on controls distinct from the flecking resulting from rust infection on darkened leaves).

Effect on infection type of continuous darkness for: One day—little change. (Notice slight hypersensitiveness at the tip of the 1-day leaf in figure 6, A). Two days—tiny pustules in nonnecrotic flecks, mingled with large pustules. The proportion of susceptible to resistant pustules on 11 leaves varied within the limits shown by the 2 "2-day" leaves in figure 6, A. These 11 leaves, therefore, were designated as "x" to "x+". Four "4" leaves also present. Three days—a range from "0"; to "x-" on 14 leaves. Four days—chiefly very small green islands with minute brownish nonnecrotic flecks; few pustules; no large pustules up to 22 days after inoculation. (Notice the 2 very sharply defined flecks toward the upper end of 1 of the 4-day leaves illustrated). Five days—as 4-day leaves. Six days—leaves killed without producing more than green blotches on very pale leaves, a few developing green islands with minute flecks. Dead when the photograph for figure 6, A was taken.

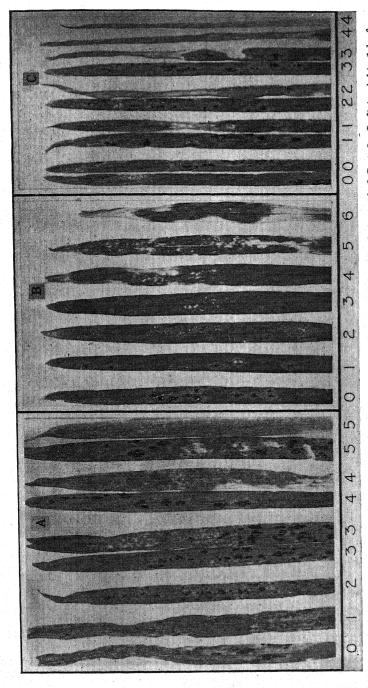
Recapitulation. The experiments just described show that the interposition of a more or less extended period of darkness at the beginning of pustule formation resulted in a loss of compatibility between the fungus and the host cells. This disadjustment was in all varieties made manifest in a pronounced modification of the infection type as read at a time when the rust on control plants had reached full development. The infection types induced on susceptible varieties were found to have all the characters ordinarily associated with standard infections of Puccinia graminis tritici on seedling hosts of various degrees of resistance. The accepted insignia of resistance thus appeared upon every tested variety ordinarily susceptible to P. graminis tritici p. f. 21 and were intensified on hosts ordinarily resistant. Host varieties that usually yield a type-"4" infection with this form were induced to bear small pustules of type-"3," green islands

which characterize the standard "2" reaction, minute pustules in flecks, sometimes sharply necrotic, which distinguish the type-"1" infection, or no pustules at all. Since type-"4" pustules were sometimes formed together with pustules of the resistant types, the resulting appearance at the time of photographing was frequently that of an "x" reaction. On host varieties in which the standard infection types are resistant, the size of pustules and of flecks was reduced with increasing duration of darkness till finally definite flecks were no longer formed.

It was clear from these experiments not only that the experimental treatment induced modification of infection type on susceptible wheat varieties to resemble that on resistant varieties but also that the degree of modification depended in any one experiment upon the duration of the period of darkness. The first modification of infection type was often in the nature of a reduction in size of pustule only. When hypersensitive tissue first appeared it generally affected only a small proportion of the infection centers and, after increasing periods of darkness, affected an increasingly large proportion of the infections on a leaf. In all experiments a period of darkness was found beyond which the proportion of infection centers accompanied by hypersensitive tissue was not increased with longer duration of darkness. In some experiments the maximum proportion was not reached until after comparatively long dark periods (Fig. 7, B, where hypersensitive tissue is shown to have increased with increasing darkness up to a 5-day period). 10 In some experiments the maximum was reached after a shorter dark period (Figs. 4, B; 6, B; 7, A; 8, A). In still other experiments, necrotic tissue appeared in association with numerous infection centers after 1 day in the dark and in no larger proportion after longer periods of darkness (Fig. 8, B). It was sometimes true of experiments on the durum varieties that 1 day in the dark was sufficient to cause the appearance of minute necrotic flecks in most of the infection centers and that longer dark periods did not increase the proportion of infection centers that were represented by flecks. This was true, for instance, of the experiment represented by figure 9, A, although secondary development of the rust (see below) had hidden the flecks on the 1-day leaves when this photograph was taken. If darkness were sufficiently prolonged in any experiment beyond the time required to produce the maximum of necrosis, the amount of necrosis might again be diminished or finally fail to appear (Fig. 4, B, 4-day leaf, or Fig. 7, B, 6-day leaf). This failure of necrosis on the plants held longest in the dark resembled the effect of darkening upon resistant varie-

10 The general necrosis at the tip and base of the 6-day leaf was distinct from that representing hypersensitive tissue. It resulted from darkening and could be seen on noninfected as well as infected leaves. In this case, only areas infected by rust remained green and turgid.

¹¹ Cf. footnote 10.



B. Kota, Exp. 62, inoculated Mar. 20, 1930, placed in dark Mar. 28, photographed Apr. 5. Left to right: 1 leaf untreated, 1 leaf each in dark 1 day, 2 days, 3 days, 4 days, 5 days, 6 days. C. Kota, Exp. 79, inoculated May 24, 1930, placed in dark May 30, photographed June 12. Left to right, 2 leaves Fig. 7. A. Arnautka, Exp. 17b, inoculated May 11, 1929, placed in dark May 17, photographed June 3. Left to right: 1 leaf untreated, 1 leaf in dark 1 day, 1 leaf 2 days, 2 leaves 3 days, 2 leaves 4 days, 2 leaves 5 days. each: untreated, in dark 1 day, 2 days, 3 days, 4 days.

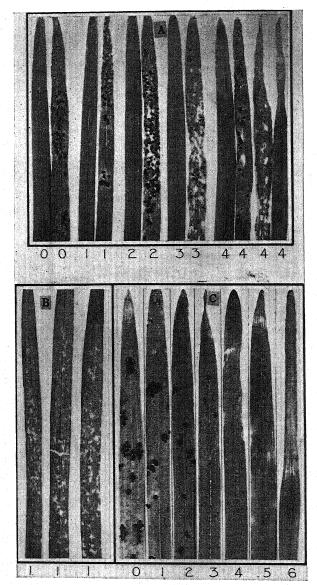


Fig. 8. A. Kota, Exp. 19, inoculated May 16, 1929, placed in dark May 22, photographed June 1. Left to right, 1 leaf each noninoculated and inoculated: untreated, in dark 1 day, 2 days, 3 days, 2 leaves noninoculated and 2 leaves inoculated in dark 4 days. B. Kota, Exp. 29, inoculated July 20, 1929, placed in dark July 24, photographed July 27. All 3 leaves in dark 1 day. C. Kota, Exp. 39, inoculated Oct. 7, 1929, placed in dark Oct. 13, photographed Oct. 26. Left to right: 1 leaf untreated, 1 leaf each in dark 1 day, 2 days, 3 days, 4 days, 5 days, 6 days.

ties that show necrosis under ordinary conditions, e.g., Khapli (Fig. 4, A). The final effect, if darkness was sufficiently prolonged (Fig. 4, B, 4-day leaf), was the total suppression of externally visible signs of the rust, infection centers being marked only by areas of the leaf that remained green after long periods of subjection to darkness.

The intermingling of infection centers that were accompanied by hypersensitive tissue with others that were not must be ascribed to heterogeneity either of the infecting mycelia or of individual host cells or groups of cells, perhaps of both. When urediniospores from either susceptible or resistant pustules experimentally produced on the same leaf were transferred to new seedlings, the controls developed susceptible infection types, but when seedlings so infected were subjected to continuous darkness resistant and susceptible infection centers were again intermingled regardless of which type served as the source of inoculum. This suggests that the heterogeneity resides in the cell population of the wheat leaf rather than in the population of rust mycelia. In any one experiment increasing duration of darkness (within the limits defined in the preceding paragraph) increased the proportion of infections accompanied by hypersensitiveness. This, again, suggests that the cell heterogeneity of the host is of the nature of differences between individual cells or groups of cells in their rapidity of progression in the dark toward whatever physiological condition of the host tissue renders it incompatible with the fungus mycelium. As time in the dark increased, this condition was reached in more and more of the host cells, and the symptoms of incompatibility accompanied more and more of the infections. Heterogeneity amongst the cells of a single leaf in respect to the time relations of changes resulting from darkness is a phenomenon already recognized in the cherry laurel (12).

The full significance of the results recorded in this section can be estimated only in conjunction with certain experimental facts to be presented below, of which the chief is the fact that in the different experiments the appearance of resistant infection centers was not dependent upon the absolute duration of darkness but was correlated with the time in the dark required under any particular set of circumstances to cause the progression of the host tissue itself through its series of visible changes to the point of death. Before considering the experimental evidence upon which such a correlation is based, however, it is necessary to digress for the purpose of describing the effects of darkness on the number of pustules and flecks formed and to pay some attention to the part played in producing the results described in the present section by the period of time during which the plants were under natural conditions between removal from the dark chamber and the reading of the results recorded.

E. Effect of continuous darkness upon the number of pustules and of total visible infection centers. The imposed experimental treatment some-

times caused a reduction in the number of uredinia or sometimes even a reduction in the number of total visible infection centers, *i.e.*, pustules plus hypersensitive flecks. Since the rust fungus had equal chances of establishing infection in all plants before they were placed in the dark chamber, this can only mean that certain nests of mycelium were prevented from developing uredinia. Some of these caused the appearance of flecks on the leaves, while some produced no externally visible signs of their presence in the tissues.

The numerical reduction of pustules and flecks was especially evident among the resistant varieties. Actual counts were made for a few experiments when it was found that the number of pustules, the ratio of pustules to flecks, and the total number of visible infection centers diminished with increase in duration of continuous darkness.

It has already been stated, in describing experiments on resistant varieties, e.g., number 72 (p. 515), that, in addition to its numerical effect on the infection centers, prolonged darkness caused a diminution in the size and the definition of hypersensitive flecks, resulting in a gradual progression toward invisibility with increase of time in the dark. It is not surprising, therefore, to find that some infections were so reduced as to be quite invisible from the exterior. This indicates that the effect of prolonged darkness upon resistant varieties constitutes a modification toward a state comparable to that designated by Stakman and Levine by the symbol "0", i.e., a modification toward a more resistant type of infection than is represented in their usual "1" reaction.

The numerical relations, where susceptible host varieties were concerned, are not so clear. In several experiments, although a diminution in the ratio of pustules to flecks resulted from subjection to darkness, no significant decrease in the absolute number of pustules or of total visible infection centers occurred. In one experiment, on the other hand, reduction in the number of pustules and the total number of visible infection centers was definitely recognizable. In this latter experiment infection was very heavy, whereas in the former experiments it was light. It is not, however, possible to decide without many more observations than I have yet made if intensity of infection determines whether reduction in the number of visible infections occurs, nor, indeed, just what is the true effect upon numerical relations of the infections when hosts susceptible to the rust form employed are subjected to darkness.

F. Secondary development of the rust. A few days after the return of experimental plants to light following a period of darkness, pustules of a susceptible type began to appear in the tissue immediately surrounding many of the modified primary infections on susceptible hosts. These I shall refer to as secondary pustules, in contradistinction to those in the area of

primary infection by the fungus. The distinction was easily made when the primary infection involved necrosis, for the margin of the primary area was then sharply defined, and the primary pustule, when one appeared, was separated by necrotic tissue from the secondary pustule. There was, therefore, no difficulty in distinguishing the two until a fairly late stage of development, when the secondary pustules sometimes completely obscured the necrotic tissue. If, however, no necrosis appeared, a difficulty arose much earlier, for it was then not so easy to determine the limits of the primary-infection area. Sometimes, as in figure 3, e, the primary and secondary pustules remained distinct for a long time, but in other instances it was impossible to tell at the time when reactions would ordinarily be read which susceptible pustules were of primary origin and which partially or wholly of secondary origin. By making daily observations on marked leaves, however, it was possible to trace the history of their pustules and so to follow the course of secondary development.

The beginnings of secondary pustules did not on any occasion become externally visible less than 3 days after the return of the host plants to light, while the usual time was about a week. In certain instances it was nearer to 2 weeks. The development of secondary pustules in 2 Arnautka experiments is illustrated in figures 3, a-e. Figure 3, a, shows a leaf held in the dark 2 days (Experiment 34) with a necrotic fleck around which secondary growth of the rust was beginning when the leaf was photographed, 15 days after inoculation and 7 days after its return to light. Four days later this infection had attained the stage shown in figure 3, b. By this time the secondary growth was almost sufficient to hide the original fleck. Unfortunately, this particular infection was lost sight of, but observation of similar cases showed that the secondary growth might eventually completely hide the original fleck, giving the appearance to one who had not followed its history, of a completely susceptible type of pustule. The appearance of secondary pustules of a susceptible nature around necrotic flecks makes doubtful the theory that an area of necrotic tissue about an infection nucleus provides an impassable barrier to the rust mycelium and thus prevents its spread. It is evident that in the case under discussion the mycelium was able to reach the green tissue beyond the fleck, possibly before the death of the tissue involved in the fleck, and that it was there able to develop normally when conditions in the outer tissues were favorable. Figure 3, c, shows a leaf from experiment 64, in which secondary pustules had already hidden most of the primary pustules but had left the nature of the lowest one, a "3" pustule with a secondary ring, still evident. This illustration was made from a photograph taken 9 days after the 1-day period of darkness had been completed. Eleven days later the infection on the same leaf had reached the stage shown in figure 3, d. The lowest pustule is here indistinguishable from the pustules of a "4" infection. Figure 3, e (Experiment 34), shows minute pustules with which no hypersensitive tissue was associated, being surrounded by secondary pustules bearing spores in abundance.

Figure 3, f, illustrates secondary growth on a Little Club leaf from experiment 66, held in the dark for 6 days and photographed 7 days after return to light. The primary infection was represented chiefly by rather poorly defined "nonnecrotic" flecks with faint green rings. Such a fleck without secondary pustules may be seen at the very base of the leaf (Fig. 3, f). The other infection centers on this leaf are represented by similar ill-defined flecks or by somewhat chlorotic areas surrounded by annular secondary pustules. These pustules later increased in size and finally completely obscured the areas of primary infection, producing an apparently susceptible infection type.

Secondary growth of the rust was partly responsible for the progressive change in the appearance of experimental plants, which has been referred to in recording the results of experiments and which made repeated observation of the plants necessary in order to be assured of recognizing the differences in infection type that could be attributed to different durations of continuous darkness in any experiment. In describing or photographing a leaf at any one time only, it is impossible to separate the effects of primary and secondary growth, and any momentary record of appearance must represent the resultant of the influence of the period of darkness acting together with the subsequent period of natural light conditions. It is in this light that photographs must be interpreted.

The rust infections on a single leaf did not always behave alike in the matter of developing secondary pustules. Sometimes resistant primary infection centers without secondary pustules appeared intermingled with those completely obscured by large pustules of secondary origin. The resulting appearance was suggestive of the standard "x" reaction, even though the primary infections had all been of a resistant type. The appearance of an "x" infection type might thus arise either from a mixture of primary pustules with and without accompanying hypersensitive tissue or from a set of uniformly resistant primary infections, some of which became obscured by secondary pustules of a susceptible type. After the "x" appearance had developed, it was not possible to tell in which way it had arisen unless one had followed the history of the individual pustules concerned.

The heterogeneity amongst primary infections in the matter of showing hypersensitive tissue or not has already been discussed, and evidence has been presented indicating that the cause of this heterogeneity probably lies within the host. Heterogeneity in the matter of sustaining secondary

growth, might also be a result of physiological heterogeneity amongst groups of host cells, of such a kind that the tissue around certain infection centers was capable of supporting secondary growth of the rust, following the treatment in the dark, while that around other infections was not. The alternative possibility must not, however, be ignored, namely, that some of the fungus mycelia within a leaf may have been killed by the effects of darkening, while others escaped destruction. This might again be referable to heterogeneity amongst host cells if the lethal effect came through the host, as is to be expected, and not directly through darkening the fungus. The question of heterogeneity with respect to secondary as well as to primary infection following subjection to darkness is one of very great interest but one which requires further experimental investigation before it can be understood.

Recapitulation. On the tissue of susceptible hosts that did not reach an irreversible state as a result of its experience in the dark, the rust developed secondary pustules of the type proper to its susceptible hosts under natural conditions in the greenhouse. This secondary development was not a change from resistant to susceptible on the part of the primary infection center itself but was an obscuring of the primary infections through envelopment by new growth. Sometimes not all of the infection centers on a leaf developed secondarily, with the result that a heterogeneous infection type was developed from a primarily resistant one.

The phenomena of secondary growth of the rust indicate that the alterations of host physiology that accompanied darkening and led to the modification of the primary-infection type were reversed upon return to light after a period of darkness that was not too prolonged, with the result that conditions approximating the original ones were ultimately reestablished except in those cells involved in the area of primary infection, where the presence of the fungus had caused an irreversible derangement or possibly the death of the protoplasm. As a result of this reversion, any subsequent growth of the rust led to a secondary infection type characteristic of the host under natural conditions. Thus it would appear that there is an association of congeniality or uncongeniality toward this form of rust with different physiological states of ordinarily susceptible host tissues, in both of which states these tissues are capable of existing under appropriate external conditions.

G. Environmental influence upon changes in the appearance of host tissues and upon changes in the infection type. Differences in response to experimental treatment greater than the individual differences amongst the leaves involved in a single experiment were encountered when a wheat variety was used at more than one time of year. In so far as these differences exceeded those in a single experiment, they must be attributed to variations in experi-

mental conditions. These were chiefly variations in environment during the growth of the plants, during confinement in the dark, and during the period following sojourn in the dark. The environment had its effect upon both type and rate of response. The age of the seedlings also affected the response to prolonged periods of darkness, and this factor is taken into consideration. The behavior of 3 varieties of wheat is described and illustrated.

- Kota was outstanding for the readiness with which it apparently responded to seasonal changes. It was used 8 times in the ordinary type of continuous-darkness experiment. Figures 7, B and C, 8, A, B, and C, and 11, C, show the response on most of these occasions.
- EXPERIMENT 12b (not illustrated)—9 pots planted Apr. 10, 1929, inoculated April 20.
 To dark chamber April 25.
 - EXPERIMENT 19 (Fig. 8, A)—20 pots planted May 6, 1929, 10 pots inoculated May 16. To dark chamber May 22. Photographed June 1.
 - EXPERIMENT 62 (Fig. 7, B)—28 pots planted Mar. 7, 1930, 14 inoculated Mar. 20 (2½-3½ in. high). To dark chamber Mar. 28. Photographed April 5.
 - Reaction of controls-"3", to "4-."
 - Effect on infection type of continuous darkness for: one to six days—green islands with necrotic halos producing a network of necrosis. Small pustules soon developed in green islands. In any one experiment, longer duration of darkness produced more necrosis, fewer and smaller pustules.
 - N.B.—Differences in the 3 experiments were quantitative only. That is, in the March experiment (No. 62) it required 4 or 5 days in the dark to produce any considerable amount of necrosis that was not overgrown by secondary pustules at the ordinary time for reading reactions (15 to 20 days after inoculation); in the April experiment (No. 12) it required only 3 days, and in the May experiment (No. 19), 2 days (Figs. 7, B, 8, A).
 - Effect on host tissue of continuous darkness for: one, two days—as controls. 12 Three, four days—in experiment 62 slightly pale; in experiments 12 and 19, pale, recovered only partially, some portions of leaves died. Five, six days—(in Experiment 62 only)—very pale, either recovered very slowly or died.
- EXPERIMENT 29 (Fig. 8, B)—40 pots planted July 13, 1929, 20 pots inoculated July 20 (3 in. high). To dark chamber July 24. Photographed July 27, 7 days after inoculation, 2 days after return of the 1-day leaves to light.
 - Reaction of controls-"3" (a few leaves "2++").
 - Effect on infection type of continuous darkness for: one day—on return to light, network of necrosis about green islands as in figure 8, B. Later "2" reaction. Two days—smaller green islands, more necrosis. Fairly numerous pustules later. Three days—necrosis, only 2 small pustules on 18 leaves. Four, five, six days—no pustules, only small, green areas on yellow background. Leaves died without further development of rust.
- Effect on tissue of inoculated plants of continuous darkness for: one day—several leaves partly killed. Two days—most leaf tips, few whole leaves, killed. Three

 12 In all experiments, unless otherwise stated, the control plants were healthy in appearance.

- days—more tissue killed, most of infected areas dead within a few days after removal from dark chamber. Four, five, six days—much tissue dead on removal from dark chamber, rest died very soon.
- Effect on noninoculated plants¹³ of continuous darkness for: one, two days—as controls. Three days—1 pot quickly recovered, 2 pots with much tissue killed. Parts of leaves not actually killed eventually recovered. Four days—only leaf bases greenish, much tissue killed, surviving parts recovered only partially. Five, six days—most of leaves were dead by 2nd day after return to light.
- N.B.—One day in the dark was sufficient to cause the appearance of an extensive green-island development in this midsummer experiment, and longer periods of darkness were correspondingly more severe in their effect on rust development than equivalent periods in any of the spring experiments described.
- 3. EXPERIMENT 94 (Fig. 11, C)—36 pots planted Aug. 30, 1930, 16 pots inoculated Sept. 5 (2½-4 in. high). To dark chamber Sept. 11, 9 pots subjected to continuous darkness, 3 control pots exposed to daylight. Photographed Sept. 21.
 - Detailed description on p. 543. In this late-summer experiment, carried out at a time when temperatures were high, short periods of darkness caused extensive necrosis, although the effect of equivalent periods of darkness was a little less severe than in experiment 29.
- 4. EXPERIMENT 39 (Fig. 8, C)—21 pots planted Sept. 28, 1929, 14 inoculated Oct. 7 (3½-4 in. high). To dark chamber Oct. 13. Photographed Oct. 26.

Reaction of controls-"3++".

- Effect on infection type of continuous darkness for: one, two days—pustules slightly smaller. Three days—small pustules, often in indefinite nonnecrotic flecks. Four days—pustules smaller, trace of necrosis as in figure 8, C. Five, six days—minute pustules in nonnecrotic flecks, or green areas. Leaves pale, much tissue killed.
- N.B.—No green islands with necrotic halos developed. With exception of trace referred to above, inoculated leaves showed no necrosis except such as occurred on noninoculated controls.
- Effect on host tissue of continuous darkness for: one, two days—as controls. Three days—slightly pale. Four days—slightly pale, a few tips dead, one leaf dead. Five days—pale, several tips dead, several leaves ½ dead. Six days—very pale, about ½ of tissue dead.
- 5. EXPERIMENT 53 (not illustrated)—32 pots planted Dec. 3, 1929, 16 inoculated Dec. 16. To dark chamber Dec. 22.
 - Very light infection. Pustules on leaves deprived of light were small, not accompanied by necrosis ("3="). Six days or more in dark killed both infected and uninfected leaves.
- EXPERIMENT 79 (Fig. 7, C)—14 pots planted May 16, 1930, inoculated May 24 (3-4 in. high). To dark chamber May 30. Photographed June 12.
 - Reaction of controls—'4-'' in appearance (from secondary growth about primary '3'' pustules). Speckling on control and 1-day leaves distinct from fleeking resulting from rust infection.
- 13 For the most part the effect of darkness was essentially the same on noninoculated controls as on infected leaves; one description, therefore, serves for both. Only in such cases as the present one, where the two were essentially different, are separate descriptions included. The descriptions are in most cases from noninoculated plants.

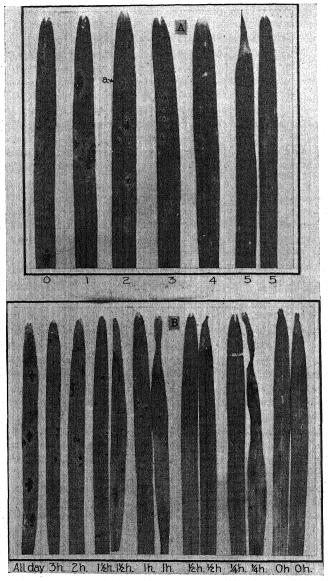


Fig. 9. A. Arnautka, Exp. 37, inoculated Sept. 29, 1929, placed in dark Oct. 6, photographed Oct. 16. Left to right, 1 leaf each: untreated, in dark 1 day, 2 days, 3 days, 4 days, 2 leaves in dark 5 days. a. Minute necrotic fleck with secondary ring forming. B. Arnautka, Exp. 36, inoculated Sept. 28, 1929, placed in dark Oct. 5, photographed Oct. 15. Left to right: 1 leaf exposed to full daylight, 1 leaf exposed daily for 3 hours, 1 leaf for 2 hours, 2 leaves for 1½ hours, 2 leaves for 1 hour, 2 leaves for ½ hour, 2 leaves for ½ hour, over a period of 10 days, 2 leaves continuously in the dark for 10 days.

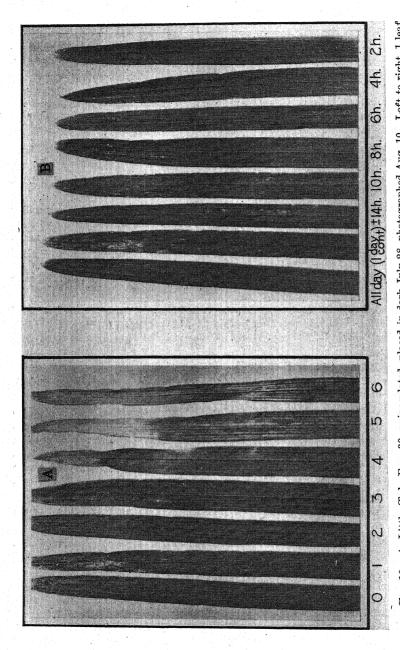
- Effect on infection type and host tissue of continuous darkness for: one day—
 ''4-'' in appearance (from secondary growth about primary ''3'' pustules). A
 few leaves partly killed. Two days—few necrotic spots, not forming network.
 Slightly pale, tips pale, several leaves partly killed. Three days—small pustules
 in nonnecrotic flecks, chlorotic areas around pustules and flecks. Upper part of
 leaves pale, most with some dead tissue. Four, five, six days—leaves died almost
 immediately after return to light.
- N. B.—Two days in the dark sufficed to cause appearance of a little necrosis accompanying rust infections; but, unexpectedly, the amount was not increased on the 3-day plants, on which nonnecrotic flecks appeared. (It is doubtful whether the large necrotic areas, such as appeared on one of the 1-day leaves in figure 7, C, can be attributed to the action of rust). It may be observed in connection with the behavior of these plants that light conditions in the greenhouse in the early part of their growth were not of the best. At the time of inoculation, a record was made to the effect that the growth was exceptionally tall and soft.
- ARNAUTKA—Arnautka was used 7 times for continuous-darkness experiments. Figures 2, 3, 7, A, and 9, A, show the response on some of these occasions.
- 1. EXPERIMENT 17b (Fig. 7, A)—12 pots planted April 30, 1929, inoculated May 11 (4 in. high or more). To dark chamber May 17. Photographed June 3.
 - EXPERIMENT 28 (not illustrated)—40 pots planted July 9, 1929, 20 pots inoculated July 16. To dark chamber July 21.
 - Effect of continuous darkness on infection type: In July, earlier appearance of extensive hypersensitiveness was evident. Loss of a day's exposure to light sufficed to produce a good deal of hypersensitive tissue, chiefly as halos about green islands. In both experiments, appearance of minute necrotic flecks soon after removal from dark was recorded. These were soon obscured by secondary growth of rust. In all except these 2 experiments, the minute necrotic flecks were the chief insignia of incompatibility; hypersensitive halos were not prevalent, although they occasionally occurred in small numbers (Fig. 2, C).
 - Effect of continuous darkness on host tissue: In both experiments, 3 days in the dark caused paling of the leaves; longer exposures increased the degree of paling. In experiment 28, 2 days in the dark sufficed to kill a good deal of host tissue, while 3 days were required in experiment 17.
 - N.B.—In one respect figure 7, A, is misleading, viz., in the effect of darkness upon the leaf tissue. Although the two 5-day leaves appear but slightly affected by the long dark period, these were the only 2 survivors out of 33 leaves. Some of the 3-day and 4-day leaves also were killed. It should be mentioned that, in selecting leaves for photographs, primary consideration was given to illustrating the effect of any treatment on rust-infection type. The effect upon general appearance of the leaves received secondary consideration.
- EXPERIMENT 31 (not illustrated)—40 pots planted July 27, 1929, 20 pots inoculated Aug. 2 (23-33 in. high). To dark chamber August 9.
 - EXPERIMENT 34 (Fig. 3, a, b, e)—40 pots planted Sept. 6, 1929, 20 pots inoculated Sept. 12 (2-3 in. high). To dark chamber Sept. 18. Photographed Sept. 27, Oct. 1.
 - EXPERIMENT 37 (Fig. 9, A)—24 pots planted Sept. 20, 1929, 12 pots inoculated Sept. 29 (2½-3½ in. high). To dark chamber Oct. 6. Photographed Oct. 16.

EXPERIMENT 58 (not illustrated)—16 pots planted Jan. 2, 1930, inoculated Jan. 13 (3-5 in. high). To dark chamber Jan. 20.

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- EXPERIMENT 64 (Fig. 3, c, d)—49 pots planted Mar. 23, 1930, 28 pots inoculated Apr. 4 (2½-3½ in. high). To dark chamber Apr. 11. Photographed Apr. 21, May 2.
- Figures 3, a, b, e, and 9, A are from Arnautka experiments of September, 1929, and represent as well the type of results obtained in January and April, 1930. In this type, loss of 1 day's exposure to light produced minute necrotic flecks, soon overgrown by secondary pustules to produce an almost or quite susceptible type of infection. Longer periods of darkness retarded growth of secondary pustules and also caused appearance of green islands, often, as in figure 2, c, around a necrotic fleck.
- N.B.—In May, 1929, 3 or 4 days in dark sufficed to kill a considerable proportion of leaf tissue; in July, 2 days were enough. In August, 3 days were required, while in September, 1929, and in January, 1930, 4 or 5 days were necessary. The April, 1930, experiment (No. 64) is exceptional in that 1 or 2 days in dark sufficed to kill several leaves in April, when a less severe effect would be expected. It may be mentioned that in this experiment even control plants grew poorly and rust infection was very light.
- LITTLE CLUB—Little Club was employed in 14 continuous-darkness experiments 2 of which are illustrated in figures 6, A, and 11, A.
- 1. EXPERIMENT 14c (not illustrated)—9 pots planted April 13, 1929, inoculated April 23. To dark chamber May 1.
 - Two days in dark chamber caused appearance of a few tiny pustules in nonnecrotic flecks among the susceptible pustules and rendered leaves slightly pale; 3 days increased the proportion of flecks and the degree of paling. Longer periods of darkness caused appearance of minute green islands in many leaves and further increased degree of paling.
- 2. EXPERIMENT 20 (Fig. 6, A)—20 pots planted May 14, 1929, 12 pots inoculated May 22 (2-3 in. high). To dark chamber May 28. Photographed June 10.
 - This experiment was similar in outcome to 14c. The only significant difference lay in the more rapid response to treatment; that is, 2 days in the dark at this time was apparently equivalent in its effect to 3 days in the earlier experiment. (p. 517).
- EXPERIMENT 15c (not illustrated)—9 pots planted April 22, 1929, inoculated May 3.
 To dark chamber May 10.
 - This experiment, intermediate in time between the 2 foregoing, responded as they did to shorter periods of darkness by production of nonnecrotic flecks intermingled with large pustules but differed from them in that green islands did not develop after longer periods in dark. Rather, the proportion of flecks to susceptible pustules increased with increasing duration of dark period. The behavior of experiment 15 was much commoner than that of the other 2 described. Indeed, it characterized most of the remaining Little Club experiments. In this experiment 4 days in dark were required to cause any noticeable degree of paling.
- 4. EXPERIMENT 24 (not illustrated)—42 pots planted June 8, 1929, 14 inoculated June 17. To dark chamber June 22.

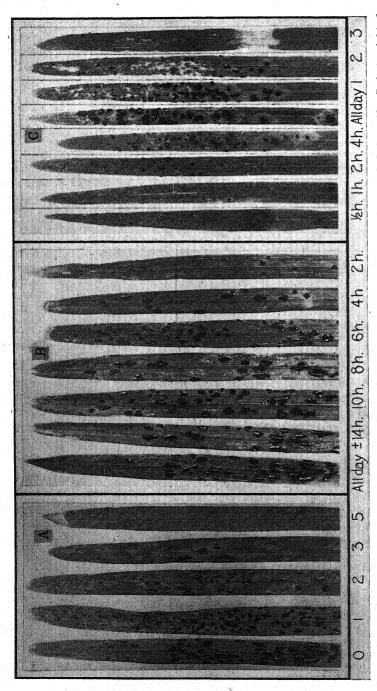
- EXPERIMENT 27 (not illustrated)—40 pots planted July 6, 1929, 26 pots inoculated July 12. To dark chamber July 17.
- EXPERIMENT 30 (Fig. 11, A)—30 pots planted July 16, 1929, 15 pots inoculated July 24 (2\frac{3}{4}-3\frac{1}{4} in. high). To dark chamber July 28. Photographed August 10.
- These summer experiments of 1929 gave results like those of experiment 15. Experiment 30 is illustrated in figure 11, A. The response in experiments 24 and 27 was not entirely confined to the appearance of nonnecrotic flecks amongst the pustules, for a comparatively small amount of the green-island type of hypersensitiveness also appeared. In experiment 30, nonnecrotic flecks (Fig. 11, A) were the only insignia of resistance.
- 5. EXPERIMENT 42 (not illustrated)—72 pots planted Oct. 10, 1929, 24 pots inoculated Oct. 20 (3\frac{1}{4}-3\frac{3}{4} in. high). To dark chamber Oct. 26.
 - EXPERIMENT 43 (not illustrated)—12 pots planted Oct. 14, 1929, inoculated Oct. 23. To dark chamber Oct. 29.
 - The 2 experiments started at the end of October were like number 30 in responding to continuous darkness by the appearance of flecks. But it required 4 or 5 days, in the dark chamber in the autumn to produce the hypersensitive flecks, while it required only 2 in July. The time required to kill any very great proportion of leaf tissue was also longer in the fall. The flecks produced in the autumn were perhaps more sharply defined than those produced in July, and some of them ultimately became necrotic.
- 6. EXPERIMENT 50 (not illustrated)—42 pots planted Nov. 20, 1929, 14 pots inoculated Nov. 29 (13-21 in.), 14 pots Dec. 1 (21-3 in.), 14 pots Dec. 3 (31-4 in.). To dark chamber Dec. 8, 10, 13, respectively. Under artificial light after removal from dark chamber.
 - EXPERIMENT 56 (not illustrated)—14 pots planted Dec. 18, 1929, 14 pots Dec. 21, 14 pots Dec. 24, 42 pots inoculated Jan. 2, 1930 (3½-4¼ in.), (3-3¾ in.), (2½-3 in.), respectively. To dark chamber Jan. 9. Under artificial light.
 - These 2 experiments conducted in December and January under artificial light each consisted of 3 lots of plants differing in age by 2 days in experiment 50 and 3 days in experiment 56. The type of rust response was much like that in October, although the flecks were not necrotic and were, for the most part, less well defined. The dark periods required to produce a visible response in rust infection were long, and host tissue was not visibly affected except by long sojourn in the dark. (p. 538).
- EXPERIMENT 63 (not illustrated)—28 pots planted Mar. 11, 1930, 14 pots inoculated Mar. 29 (2-2½ in. high). To dark chamber April 4.
 - EXPERIMENT 66 (Fig. 3, f)—28 pots planted Mar. 31, 1930, 14 pots inoculated Apr. 10 (2-2\frac{3}{4} in. high). To dark chamber April 16. Photographed April 29.
 - The early spring experiments of 1930 did not differ greatly from those conducted during the winter under artificial light. Rust response to experimental treatment consisted in the production, after several days in the dark, of nonnecrotic flecks surrounded by secondary circles of sporulating pustules (Fig. 3, f). In experiment 66 one of the 6-day leaves showed no secondary growth of rust but produced, rather, a small pustule in a necrotic fleck (a good "1" type). Five days of continuous darkness were required in both experiments to kill any considerable proportion of host tissue.



each: untreated, in dark 1 day, 2 days, 3 days, 4 days, 5 days, 6 days. B. Little Club, Exp. 30, uninoculated, placed in dark July 28, photographed Aug. 10. Left to right, I leaf each, exposed to daylight: all day, about 14 hours, 10 Fig. 10. A. Little Club, Exp. 30, uninoculated, placed in dark July 28, photographed Aug. 10. Left to right, 1 leaf hours, 8 hours, 6 hours, 4 hours, 2 hours, over a period of 13 days.

- EXPERIMENT 74 (not illustrated)—13 pots planted April 8, 1930, inoculated April 19 (2½-3 in. high). To dark chamber April 27.
 - In experiment 74 the flecks were also of 2 kinds, (1) nonnecrotic and rather indefinite or (2) conspicuous and necrotic. This experiment showed again the more rapid effect on appearance of leaf tissue that characterized the spring and summer experiments of 1929.
- 9. EXPERIMENT 71 (not illustrated)—42 pots planted April 12, 1930, 14 pots lot (b) inoculated April 24 (2½-3¼ in.), 14 pots lot (c) April 26 (3-3¾ in.). To dark chamber April 30, May 2, respectively.
 - The slightly older plants (c) reached the point of irreversibility (p. 502) faster than those 2 days younger and, correspondingly, required a shorter time in the dark to cause the production of hypersensitive flecks. Here the flecks were nearly all necrotic, large and well defined (Fig. 2, f). In 71b secondary growth made more progress than it did in 71c, but necrotic flecks were present here also. The severity of the effect of continuous darkness on rust infection increased in this experiment beyond the degree it had reached since the summer of 1929. This indicates a return to the conditions of the experiments of the previous late spring and summer and agrees with the behavior of the other varieties in showing, on the whole, more rapid change in the general appearance of host tissues and greater modifications of rust-infection type resulting from subjection to darkness in summer than from equally long subjection to darkness in winter. (p. 537).

Recapitulation. a. Effect upon type of infection. Each variety that was used several times for experimentation responded by the production of more than one infection type. In 4 out of 7 Kota experiments only green islands appeared, but in others green islands were, for the most part, replaced by nonnecrotic flecks (Figs. 7 and 8). Arnautka and Little Club produced minute green islands and extensive hypersensitive tissue under certain conditions (figs. 6, A, and 7, A). The minute flecks of the type shown in figure 9, A, were the only other symptoms of resistance displayed by Arnautka. Little Club also developed the Khapli type of infection (Fig. 2, f) and on many other occasions developed hypersensitive flecks, which did not resemble a standard Khapli infection so closely (p. 499; also Fig. 11, A). In certain experiments carried out in the fall or winter, e.g., Kota, experiment 39 (Fig. 8, C) and experiment 53, no sharply hypersensitive tissue appeared after any period of darkness. This further emphasizes the effect of environment on the type of infection resulting from the subjection of seedlings to darkness. Environmental conditions during growth of the seedlings, the temperature prevailing in the dark chamber, and the conditions after the dark period ends must all be considered as possible factors influencing type of infection. It is probable that the environment during each of these three periods played its part in producing the protoplasmic states that determined the infection type on various occasions with the rust-host combinations involved.



Fro. 11. A. Little Club, Exp. 30, inoculated July 24, 1929, placed in dark July 28, photographed Aug. 10. Left to right, 1 leaf each: untreated, in dark 1 day, 2 days, 3 days, 5 days. B. Little Club, Exp. 30, inoculated July 24, 1929, placed in dark July 28, photographed Aug. 10. Left to right: the same normal leaf as appears in figure 11, A, i.e., exposed to daylight all day, 1 leaf each exposed to light daily for: about 14 hours, 10 hours, 8 hours, 6 hours, 4 hours, 2 hours, over a period of 13 days. C. Kota, Exp. 94, inoculated Sept. 5, 1930, placed in dark Sept. 11, photographed Sept. 21. Left to right, 1 leaf each: exposed to light daily for: 4 hour, 1 hour, 2 hours, 4 hours, all day; in continuous darkness for: 1 day, 2 days, 3 days.

There are certain evidences, also, of the importance of varietal or specific characters acting as internal factors in determining the type of infection under the experimental treatment here employed. In the first place, when, on a few occasions, two or three varieties were used for simultaneous experimentation, varietal differences showed up in seedlings of the same age and under identical environmental conditions. For example, in experiments 12a and b, inoculated April 20, 1929, the Little Club reactions following subjection to darkness were of the "x" type, while Kota produced its characteristic green islands. Kota's strong tendency toward green islands with a network of necrotic halos on seedlings subjected to darkness was, indeed, unique, although other varieties, including Little Club, developed green islands on certain occasions. Again, the particular type of necrotic fleck illustrated in figures 2, g; 2, h, and 9, A, might be called the durum type, for it occurred upon all durum varieties tested and was not encountered on any other variety. Furthermore, certain indications were found that Little Club required a longer period of darkness than any of the eight hosts under simultaneous test with it at one time or another, either to produce visible changes in host tissues or to bring about a change from compatibility to incompatibility with the rust fungus. Such indications are of interest because Little Club is more nearly completely susceptible to Puccinia graminis tritici as a whole than is any other of the differential host varieties. Further details concerning such evidence are omitted because of lack of space and because evidence of a more conclusive nature is being developed through experimentation in a controlled environment. Discussion of the matter is, therefore, reserved until the evidence is more complete.

b. Effect upon time relations. The rapidity of change in appearance of host tissues in the dark chamber and also the duration of darkness required to cause the subsequent appearance of a resistant infection type on any particular susceptible host variety differed under different sets of circumstances. The data concerning time relations in different experiments, as relating to the three varieties discussed in this section, are assembled in table 2. Column IV of this table presents the number of days' darkness required in each experiment to cause the subsequent death of any considerable proportion of host leaves, that is, to cause the death of more than the tips of a representative number of leaves in the pots receiving any particular treatment.¹⁴ This time interval may be looked upon as a rough index to the rapidity of progression of dark metabolism to a point of irreversibility (p. 502). In many experiments values for noninoculated plants were obtained as well; but, since these are virtually the same as for inoculated plants, they are omitted. Column V represents the number of days in the

14 Death subsequent to subjection to prolonged periods of darkness is progressive from tip to base (p. 502).

TABLE 2.—A summary of experimental results showing correlation of the duration of darkness required to kill any considerable proportion of host tissue with the duration of darkness required to replace campatibility by incompatibility with Puccinia graminis tritici p.f.21

Variety	Exp. no.	Date of	No. of days of e	continuous darkness uired to:
, azzorj		inoculation	Kill host tissuea	Cause incompatibility
Te	II	III	IV	V
Kota	29	July 20, 1929	1	1
IXUta	79	May 24, 1930	$\overline{2}$	1 2
	19	May 16, 1929	$\ddot{3}$	$\frac{1}{2}$
	12b	Apr. 20, 1929	4	3
	62	Mar. 20, 1930	5	4 or 5
	39	Oct. 7, 1929	4 5 5	_b
	53	Dec. 16, 1930	6	_b
Arnautka	28	July 16, 1929	2	1
	31	Aug. 2, 1929	3	2
	17b	May 11, 1929	3-4	2
	58	Jan. 13, 1930	4	3
	37	Sept. 29, 1929	5	3
	34	Sept. 12, 1929	5	3–4
	64	Apr. 4, 1930	1-2d	3
Little Club	30	July 24, 1929	3	2
	71cc	Apr. 26, 1930	3	4
	24	June 17, 1929	3–4	2
	20	May 22, 1929	4	2
2004		July 12, 1929	4	2–3
	74	Apr. 19, 1930	4	4 v. little
	71bc	Apr. 24, 1930	4 5	5 5
	42w	Oct. 20, 1929	5	5 v. little
	56ac 63	Jan. 2, 1930	5	
	66	Mar. 29, 1930	5	5-6 6
	50bc	Apr. 10, 1930 Dec. 1, 1929	5–6	5 v. little
	42u	Oct. 20, 1929	5-0	5 v. 11tile
	50ac	Nov. 29, 1929	6 or more	5
	43	Oct. 23, 1929	more than 6	4
	56bc	Jan. 2, 1930	6-7	6 v. little
	56cc	Jan. 2, 1930	more than 7	6 v. ''
Little Club	50ac	Nov. 29, 1929	6 or more	5
	50b.	Dec. 1, 1929	5-6	5 v. little
	56c	Jan. 2, 1930	more than 7	6 v. "
	56b	Jan. 2, 1930	6-7	6 v. "
	56a	Jan. 2, 1930	5	5 v. "
	71b	Apr. 24, 1930	4	5
	71c	Apr. 26, 1930	3	4

^a By "kill host tissue" is meant the killing of more than the tips of a representative number of leaves. By "cause incompatibility" is meant the production of hypersensitive tissue that remained visible 15-20 days after inoculation.

b No hypersensitive tissue appeared.

c In experiment 50a the plants were 9 days old when inoculated; 50b, 11 days old. In experiment 56a the plants were 15 days old; 56b, 12 days old; 56c, 9 days old. In experiment 71b the plants were 12 days old; 71c, 14 days old.

d See note on experiment 64, on page 530.

e The column numbers are used merely for convenience in referring to the table.

dark required in each experiment to cause a substantial failure of compatibility between the fungus and its host. Such a failure was regarded as having occurred when hypersensitive tissue (whether in the form of flecks or of halos about green islands) appeared in sufficient quantity to escape being totally obscured by secondary development of the rust 15 to 20 days after inoculation.

The time interval in column V is an index to the rapidity of change from compatible to incompatible relations between host and fungus, which provides a basis of comparison with the index to rapidity of metabolic change in the host presented in column IV. These indices make no claim to mathematical precision but are adequate for purposes of the present discussion. Rapidity of progression of dark metabolism to a state of irreversibility (resulting in the death of the tissue) and rapidity of change from compatibility to incompatibility are used in a sense reciprocal to the duration of darkness required to effect these changes. They bear no implications as to the manner in which they were brought about.

Considering the nature of the indices, the degree of correlation between the duration of darkness required to affect the host tissues irreversibly and that required to disturb the compatible relations between fungus and host seems highly significant. The numerical correlations are not exact, nor could they be expected to be. But when the experiments were arranged in such order that the values for each variety in column IV were in ascending order of magnitude, then, with only one or two exceptions, the values in column V were, also, in ascending order of magnitude. Where differences in column IV were small, a correlation with column V was not revealed; but, where differences in column IV were substantial, column V did not fail to show correlative differences.

It will be observed that, in general, the period of darkness requisite for the production of resistant infection types on these susceptible varieties was shorter in summer when both greenhouse and dark-chamber temperatures were high and change in the appearance of host tissue was rapid and was longer in winter when both greenhouse and dark-chamber temperatures were comparatively low and change in the appearance of host tissues was slower. An additional factor was found to be of some importance in affecting rapidity of change in general appearance of host tissues and in infection type. This was the age of the experimental plants. The effect of this factor is shown at the bottom of table 2 in the values given for experiments 50, 56, and 71. These three experiments will come under discussion later. To be remarked now is the strongly suggested correlation, whatever the factors determining it, between the duration of darkness required for the disturbance of compatibility between host and fungus and the duration of darkness required so to affect the host tissues as to cause death, although

the plants were returned to natural light conditions at the end of the dark period. The significance of such a correlation can only be that there is a common factor in the causation of the two effects. That this is a metabolic factor belonging to those processes in host metabolism that continue in the dark rather than to those that cease in the absence of light is a conclusion to which we are led by the following considerations: 1. More than a simple retardation of fungus growth resulted from the period of darkness; its relationship with the host tissue was so changed that all the signs of incompatibility appeared on hosts ordinarily entirely compatible with the fungus. 2. Appearance of symptoms of incompatibility on any susceptible variety did not depend upon the absolute duration of darkness. It was associated, rather, with the duration of darkness, widely different in different experiments, necessary to bring about an irreversible change in the appearance of the host tissues.

There is no implication that any definite appearance of a leaf is to be associated with loss of congeniality toward rust infection; indeed, the symptoms of incompatibility very often appeared in leaves not visibly affected in any way by their sojourn in the dark. It is intended here merely to imply that a darkened leaf, which passes rapidly through its series of visible changes, is passing rapidly through its course of metabolic changes, the early stages of which precede visible changes, and to point out that rapidity of this progression was associated in my experiments with rapidity of change from compatibility to incompatibility with the rust pathogen, whereas slow progression was associated with a slow change to incompatibility.

H. Effect of slight differences in the age of the host upon changes in the appearance of host tissue and upon changes in the infection type. Three experiments originally planned to detect, if possible, the causes of individual variation amongst leaves or amongst pots receiving the same experimental treatment substantiated the suggested correlation between metabolic change in darkened host tissues and disturbance of compatibility with the rust fungus employed. These three experiments dealt with plants differing but a few days in age when inoculated. Little Club wheat was used for all of them.

Two of the three experiments were carried out in the winter of 1929–30 on plants inoculated at the ages of 9, 11, 13 days for experiment 50 and of 9, 12, 15 days for experiment 56. The effects of continuous darkness for periods of from 1 to 7 days were much alike as to rust-infection type on plants of all the ages tested. Indications of a slightly more severe effect on the older plants, however, were found. The tissues of the older host plants showed more rapid progression through their characteristic series of visible changes in the dark than did those of the younger plants.

The third experiment, No. 71, was carried out in the spring of 1930. Plants were inoculated at the ages of 12 and 14 days. Necrotic flecks (Fig. 2, f) developed after a shorter period of darkness and more abundantly after equivalent periods of darkness on the older plants than on the vounger (p. 533). At the same time there resulted from subjection to darkness a more rapid progression to death of the tissues of the older plants (Table 2). Removal of the endosperm at the time of inoculation from 1/2 the plants receiving each treatment revealed no differences resulting from this operation. This suggests that the endosperm was not responsible for whatever change with age in the physiological constitution of the seedlings accounted for the changes in response to darkness, for a difference of 2 days in the age of the seedlings was correlated in this experiment with more pronounced differences than were seen in either of the winter experiments, both as to rapidity of progression of the leaf tissues to death following subjection to darkness and as to rapidity of change in the dark from compatibility to incompatibility with the rust pathogen involved.

It seems important to know the nature of the rapid change in physiological constitution of the seedlings that must underlie these differences. the first place, the differences that appeared to depend on slight inequality in the age of the seedlings were of the same sort as occurred amongst individual plants throughout the experiments, and it is possible that variation in the rates of physiological aging of the seedlings used for a single experiment can be shown to account for at least a part of the individual variations observed. 15 In the second place, this rapid change in physiological constitution of the seedlings affords further instances of a correlation of the duration of darkness required to cause death of host tissue with the duration required to disturb compatible relations with the rust form involved and introduces age of tissue as a possible factor in determining both of these quantities. This relation of age of host to effect of darkness on host tissue and on rust infection suggests that experimentation with plants of wider age differences might provide valuable information concerning the possible existence and nature of a physiological element in mature resistance. It may be pointed out that the age of the tissue or organ concerned is known to affect the time relations and even the course of dark metabolism in other plant tissues (12), (2), and (3).

II. EXPERIMENTS ON SEEDLINGS WITH SHORT DAILY EXPOSURES TO LIGHT

A. Conditions of experimentation. Plants were inoculated as usual and the rust was allowed to establish itself before experimentation began.

15 Preliminary experiments in which plants were grown in water culture instead of in soil and one experiment for which Little Club seeds were selected within a narrow range of weight failed to reduce individual variation or to reveal any indications of its cause.

When infection flecks first appeared, the experimental pots were transferred to the dark chamber whence they were transferred to the greenhouse bench for the desired number of hours each day. This was continued either throughout the period of development of the rust or, for the sake of comparison with this treatment, for a few days only, after which the plants were left continuously on the greenhouse bench. The exposures to light usually were arranged so that they centered around noon. For instance, a 10-hour exposure began at 7:00 a. m. and ended at 5:00 p. m. The longest and shortest exposures were sometimes exceptions to this. For instance, one exposure was from 7:00 a. m. to dark, or about 9:00 p. m.; \(\frac{1}{4}\)-hour exposures were usually from 11:15 to 11:30 a. m.; and \(\frac{1}{2}\)-hour exposures from 11:00 to 11:30 a. m.

B. Effect of short daily exposures to light upon the appearance of the host tissues. Figure 10, A and B, compares the effects of continuous darkness and of short daily exposures on Little Club seedlings inoculated in July, 1929. A detailed description of the effect of the two experimental treatments in this particular case is to be found on page 542. It is obvious (Fig. 10, B) that interruption of darkness by even a short light period once in 24 hours prevented the progress of dark metabolism to the point where any visible change in the leaves appeared. Even 2 hours of light a day over a period of 13 days sufficed to prevent the slightest paling of the leaves in 3 out of 4 pots so treated. Reference to the descriptions of other experiments of the same nature involving other wheat varieties as well as Little Club (pp. 542-545) will show that 4-hour light periods daily were in nearly all cases sufficient to prevent any visible change in the host tissues. Usually shorter periods of exposure eventually caused progressive paling, loss of turgor, and finally death of the tissues. The same gradient in rapidity of change from tip to base of the leaf was found here as was recorded for plants held continuously in the dark. Even short exposures to light, however, delayed these visible changes in the leaves considerably beyond the time when they appeared on corresponding leaves held continuously in the dark.

C. Effect of short daily exposures to light upon rate of rust development. Table 3 has been prepared in the same way as was table 1. In it are collected the data on rate of development of pustules for all experiments on short daily exposures to light. As before, column IV represents the number of days required for the first appearance of infection flecks after inoculation, and the remaining columns the number of days from the appearance of infection flecks until the majority of pustules were formed as swellings under the epidermis. The most striking information contained in the table is that the rust developed just as fast with a very few hours' exposure to daylight each day as it did when the plants were exposed all day. Kota,

TABLE 3.—A summary of experimental results concerning the effect of short daily exposures to light upon the rate of pustule development of

Puccinia graminis tritici p.f.21 on 5 differential host varieties

				Lapse c	of time	in days ad form	in days between the first appearance of infection fleeks and the stage when had formed pustules, with daily exposures to light of the following durations:	the fir	st appe h daily	arance exposu	of infe res to l	ection flight of	ecks an the fol	d the s llowing	tage wh duratio	nen most	Lapse of time in days between the first appearance of infection flecks and the stage when most of these had formed pustules, with daily exposures to light of the following durations:
uriety	Exp. No.	Date of inoculation	oculation to appearance of flecks	All	8:00 a.m. dark	9:00 a.m. dark	10 hr. 8 hr.		6 hr.	4 hr.	3 hr.	2 hr.	1½ hr.	1 hr.	½ hr.	4 hr.	0 hr.
I.	П	III	ΛI	Δ	VI	VII	VIII	IX	×	IX	ХП	XIII	XIV	ΛX	XVI	XVII	XVIII
e Club	30	July 24, 1929	4 8	ന ന	က	æ:	מי	က :	၁ :	o 4 5-4	: :	5		7-9	1 1		1
	41 94	Sept. 5, 1929	9	2 CJ	1 1	: i			:	c 1	:	67	: 3	01	2-3	:	
utka	36	Sept. 28, 1929	7	3-4				:	:	4	4	¥ 4	Ž :	5 4	1 1		
corn	45 88	Nov. 8, 1929 Sept. 30, 1929	9	 5 or	: :	1 1	: 1	: :	: 1	;		. 1	·	ı	ı	ı	:
pli	35	Sept. 14, 1929	∞	more 4	. 1:	4		4	4	4-5	:	1		1	ı	:	
a A serie b A minu c The col	s of dots is sign (-	a A series of dots () signifies that the data required to fill a certain space is b A minus sign (-) signifies that few or none of the infection flecks develope of The column numbers are used merely for convenience in referring to the table.	the data required to fill a certain space in the table are lacking. w or none of the infection flecks developed pustules as long as the plants were kept under observation or until they died. ly for convenience in referring to the table.	ed to fil e infect e in ref	l a cert ion flec erring t	ain spacks develor the tal	se in the oped pus ble.	table a	re lacki	ng.	lants w	rere kep	t under	· observ	ation o	r until t	hey died.

inoculated in September (Experiment 94), showed no retardation of pustule development when the exposure was reduced to 1 hour per day, and retardation was doubtful even with ½ hour exposure. Little Club, inoculated in July (Experiment 30), showed the first retardation when the exposure was reduced to 2 hours per day, while in October (Experiment 41) plants exposed 4 hours a day required 1 or 2 days longer to develop pustules than those exposed all day. Arnautka (in Experiments 36 and 45, both in the late fall or winter) showed no retardation when the exposure was reduced to 1 hour a day. With shorter exposures pustule development was almost entirely suppressed. As with Arnautka, the effect of very short exposures to light on Einkorn and Khapli was to suppress pustule development; and, since only short exposures and full daylight were used with Einkorn, no information concerning rate of development is available. The Khapli experiment shows that daily exposures as short as 4 hours did not influence the rate of development of the rust on this variety.

D. Effect of short daily exposures to light upon the infection type. Experiments involving Little Club, Arnautka and Kota all failed in the production of well-defined hypersensitive tissue following short daily exposures of the experimental plants to light. This offers a sharp contrast to experiments described here for the sake of comparison, wherein continuous darkness, as in experiments recorded earlier, caused the appearance of sharply hypersensitive tissue on these host varieties. Resistant varieties responded to short daily exposures to light much as they did to continuous darkness, that is, by reduction in pustule size and number and in definition of flecks.

LITTLE CLUB, EXPERIMENT 30 (Figs. 10 and 11). Sixty pots planted July 16, 1929, 30 pots inoculated July 24 (2\frac{2}{n}-3\frac{1}{4} in. high). Infection flecks appeared July 28 and all but control pots placed in dark chamber. Some were left in dark chamber continuously for periods of 1 to 7 days, then returned to the greenhouse; that is, they were subjected to continuous darkness. Others were removed from dark chamber daily for periods of 2, 4, 6, 8, 10, and about 14 hours and were kept in dark the rest of the time; this treatment was continued for 13 days. Photographed Aug. 10, the 13th day after initiation of treatment and 17th day after inoculation.

Effect on infection type:

Continuous darkness (Fig. 11, A):—Nonnecrotic flecks even after 1 day in dark; increased in proportion to number of pustules with increasing periods of darkness.

Short daily exposures to light for 13 days (Fig. 11, B): Exposed: 14 ±, 10, 8, 6, 4 hours daily—no effect on the appearance of the rust. Two hours daily—reduction in size of pustules. No change in infection type. No hypersensitiveness. (The ill-defined whitish areas on some leaves were not effect of rust infection. They were present on many noninoculated leaves and can be seen in figure 10. They happen to be fairly numerous on the leaf kept in the dark 1 day. These were readily distinguishable from the nonnecrotic flecks reported on leaves subjected to periods of continuous darkness).

Effect on host tissue:

Continuous darkness (Fig. 10, A): 1, 2 days—no significant change in appearance.

Three days—a few leaves were irreversibly affected (p. 502), most of them not seriously damaged. Four days—much tissue killed, surviving tissue quite pale.

Five, 6 days—most of the leaves killed.

Short daily exposures to light for 13 days (Fig. 10, B): Exposed: 14 ±, 10, 8, 6, 4 hours daily—no effect on appearance. Two hours daily—in 1 pot, no effect, in 1 pot the leaves became pale and flaccid after 8 days of this treatment and finally died. (The 2 pots of inoculated plants exposed to light 2 hours daily behaved as did the first pot mentioned; that is, they showed no deleterious effect of prolonging their nightly periods of darkness to 22 hours, thus shortening their daily light periods to 2 hours).

LITTLE CLUB, EXPERIMENT 41 (not illustrated)—32 pots planted Oct. 8, 1929, 16 pots inoculated Oct. 17 (2½-3 in. high). Exposures 4, 2, 1½, 1, ½, ½ hours per day—2 types of experimental procedure: (1) Short daily exposures continued 9 days and then plants returned to natural conditions. (2) Short daily exposures continued 19 days.

Effect on infection type of both treatments:

Exposed to light: All day—''4+''. Four hours daily—no change. Two hours daily—on some leaves no change, except in reduction of size of primary rust pustules; on some leaves some effect on host tissue occurred that might be classed as chlorosis, it was of a diffuse type, and not sufficiently defined to be classed as hypersensitive tissue. 1½, 1, ½ hours daily—pustules smaller, no definite flecks. One-fourth hour daily—on 1 leaf so treated for 9 days 1 rather definite non-necrotic fleck developed by the 26th day after the initiation of the treatment. (Throughout these experiments with short daily exposures to light, leaves exposed only ½ hour daily did not behave very differently from those held continuously in the dark. Such a short exposure evidently did not allow for much physiological readjustment in leaf tissue. The same applies to behavior, on some occasions, of leaves exposed ½ hour daily.)

N.B.—Throughout the experiment, the difference between plants confined to short periods of light for 9 days and those so treated for 19 days lay entirely in the presence of secondary rust growth on the former and its absence on the latter 19 days after transfer of the plants to the dark. Seven days later, secondary growth was present on the 19-day-treated plants as well, although after exposures less than 2 hours a day such growth did not advance very far.

Effect on host tissue:

Nine-day treatment: Exposed: 4 hours daily—like plants exposed all day. Two, 1½ hours daily—became slightly pale. One hour daily—became pale, a good deal of tissue died. One-half, ¼, O hours daily—pale, died a few days after return to natural light conditions.

19-day treatment: Exposed: 4 hours daily—like plant exposed all day. Two, 1½ hours daily—became slightly pale. One hour daily—pale, several tips or ends died. One-half, ½, 0 hours daily—died after about 14 days.

Kota, Experiment 94 (Fig. 11, C)—36 pots planted Aug. 30, 1930, 16 pots inoculated Sept. 5, (2\frac{1}{4} - 4 in. high). To dark chamber Sept. 11. Nine pots subjected to continuous darkness, 4 pots held in dark chamber for 11 days with daily exposures to

light of 4, 2, 1, $\frac{1}{2}$ hours per day. Three control pots exposed to natural light conditions. (All but the 16 pots inoculated were destroyed by a squirrel). Photographed Sept. 21.

Reaction of controls: "3+" to "3+".

Effect on infection type:

Continuous darkness: 1 day—2 out of 3 leaves with green islands and necrotic halos.

Two days—more necrosis as halos about green islands. Three days—as 2 days, but fewer pustules and fewer green islands. Four, 5 days—a few green areas with minute flecks, died very soon after removal from dark chamber.

Short daily exposures for 11 days: Exposed: 4 hours daily—''3+''. Two hours daily—''3''. One hour daily—''3=''. Some minute pustules in green areas; no necrosis. One-half hour daily—minute pustules in green areas, no necrosis.

Effect on host tissue:

Continuous darkness: 1 day—as controls. Two days—slightly pale. Three days—pale, a few tips dead. Four, 5 days—pale, upper parts almost white, soon dying.

Short daily exposures for 11 days: Exposed: 4 hours daily—as controls. Two hours daily—2 leaves as controls, 1 pale and flaccid. One hour daily—pale, infected areas green, 2 tips flaccid. One-half hour daily—completely etiolated, infected areas green, becoming flaccid.

ARNAUTKA, EXPERIMENT 36 (Fig. 9, B)—30 pots planted Sept. 19, 1929, 15 pots inoculated Sept. 28 (2½-3½ in. high). (Some of the plants were reinoculated the next day because they were flooded in the inoculation chamber, and the spores were possibly washed off the leaves). To dark chamber Oct. 5. Kept in the dark as long as they survived with daily exposures to light of 3, 2, 1½, 1, ½, ¼, 0 hours. Control pots exposed all day. Photographed Oct. 15, 10 days after initiation of treatment.

ARNAUTKA, EXPERIMENT 45 (not illustrated)—24 pots planted Oct. 29, 1929, inoculated Nov. 8. To dark chamber Nov. 14. One-half of experimental pots kept in dark chamber for 5 days, ½ for 14 days, with daily exposures to light of 4, 2, 1, ½, ¼, 0 hours. Control pots exposed all day.

Effect on infection type:

Arnautka provided a very striking example of the different effects of continuous darkness and short daily exposures to light. Figure 9, A illustrates experiment 37 (p. 529), a continuous-darkness experiment carried out almost simultaneously with experiment 36, the short-exposure experiment illustrated in Figure 9, B. (Inoculations were 1 day apart). The outstanding feature of the infection on leaves exposed to light for short periods daily is the total absence of necrotic flecks. A reduction in the size of the pustules resulted from this treatment, and in the 1hour leaves a diffuse nonnecrotic effect upon the host tissue appeared (Fig. 9, B). On the other hand, many of the leaves subjected to continuous darkness in experiment 37 bore sharply necrotic flecks. Figure 9, A does not show such flecks on 1-day leaves, but they were present earlier on these leaves and were overgrown by secondary pustules before the photograph was taken. On 2-day leaves (at a) and on 3-day and 4-day and 1 of the 5-day leaves they can be seen. It will be observed from figure 9, B that leaves of experiment 36 exposed continuously to dark produced no necrotic flecks. These leaves were, however, 10 days in the dark before being photographed. In a previous section, it was pointed out that necrotic flecks do not appear after very long periods in the dark. Even Khapli

lost them in experiment 22 after 5 days in the dark (Fig. 4, A), and Figure 9, A, shows that, in experiment 37, even after 5 days of continuous darkness, Arnautka did not produce necrotic flecks on all of its leaves. The same thing was demonstrated by experiment 45, a 2nd short-exposure experiment with Arnautka, in which inoculations were made on Nov. 8, 1929. On this occasion, leaves kept continuously in the dark for 5 days produced necrotic flecks, while those kept there for 10 days produced none. The leaves were dead before 14 days were over, but a few survived for 10 days. Plants of the same experiment exposed to light for daily periods of ½, 1, 2, 4 hours, respectively produced no necrotic flecks, whether this treatment was continued for 5 or 14 days. In development of pustules they resembled experiment 36 very closely. Necrotic flecks were according to these observations, characteristic of Arnautka plants only after sufficient continuous sojourn in the dark. When darkness had been too prolonged (more than 5 days) or too short (less than 24–36 hours), the flecks did not appear.

Effect on host tissue:

Experiment 45:

5-day treatment: Exposed to light: 4 hours daily—no effect. Two, 1 hour daily—slightly pale, recovered. One-half, 4 hour daily—pale, recovered color partially, most tissue died. Subjected to continuous darkness for 5 days—pale, partially recovered, much tissue died.

Fourteen-day treatment: Exposed to light: 4 hours daily—very slightly pale.

Two hours daily—slightly pale, ends dead. One, ½ hour daily—very pale, much dead tissue. One-fourth hour daily—nearly all dead. Subjected to continuous darkness—all dead after 14 days, a few survived 10 days.

Experiment 36:

Exposed to light: Three, 1½ hours daily—became pale after about 10 days. One, ½ hour daily—became very pale, much tissue died.

EINKORN, EXPERIMENT 38 (not illustrated)—36 pots planted Sept. 21, 1929, 17 pots inoculated Sept. 30 (3½-4 in. high). To dark chamber Oct. 7. Fourteen inoculated and 14 noninoculated pots subjected to continuous darkness, 3 inoculated, and 3 noninoculated pots kept in dark with daily exposures to light of 2. 1, ½ hours.

KHAPLI, EXPERIMENT 35 (not illustrated)—28 pots planted Sept. 6, 1929, 15 pots inoculated Sept. 14 (4-5 in. high). To dark chamber Sept. 22. One-half kept in dark 8 days, ½ 18 days, with daily exposures to light of about 14, 8, 6, 4, 2, 1, ½ hours.

Effect on infection type and host tissue:

Einkorn and Khapli are both highly resistant to form 21 and under conditions of short exposures to light responded much as they did to periods of continuous darkness, that is, by reduction in 3 respects, proportion of flecks that bore pustules to those that did not, size of flecks, and definition of flecks. Khapli plants exposed to light ½ hour daily were much like the 5-day plants of Figure 4, A. Plants exposed 1 hour daily produced almost no pustules. They presented an appearance similar to that of the 4-day plants in figure 4, A, while the 2-hour leaves were much like those kept 2 or 3 days continuously in the dark. Exposure to light for 8, 6, and 4 hours, respectively, produced an infection not observably different from plants allowed to develop under greenhouse conditions. In general, the same statement is true for Einkorn as for Khapli. As with the other varieties discussed, only in plants receiving the shortest light exposures did changes in the appearance of the host tissue occur.

Recapitulation. Under the conditions of these experiments, long periods of darkness when interrupted frequently by short periods of light did not produce the same effect as shorter periods of uninterrupted darkness. Four-hour exposures to light daily sufficed to prevent any marked differences in either rate or final appearance of rust development from those observed under natural conditions of illumination in the greenhouse. Shorter daily exposures to light caused retardation of development and reduction in pustule size but no distinctly hypersensitive tissue, although continuous subjection to darkness for 36 hours, or, in some instances longer, caused the appearance of well-defined hypersensitive flecks or halos on plants quite comparable to those receiving the short exposure treatment. Four-hour light periods daily were sufficient to prevent any effect of prolonged darkness on the external appearance of the leaves, and shorter light periods delayed the paling and subsequent changes resulting from darkening of the seedlings.

Except on the theory that a disadjustment of compatible relations between host and fungus is dependent upon the metabolic sequence in darkened tissue, it would be very surprising that plants continuously in the dark 2 nights and 1 day should develop even a slight amount of definitely hypersensitive tissue, while corresponding plants almost continuously in the dark 13 nights and days, with only a 2-hour exposure to daylight once in 24 hours, should develop none. The plants that were in the dark 2 nights and 1 day were subjected to a longer continuous period of darkness than any of those exposed to light once in 24 hours, although the total sojourn of the latter in the dark greatly exceeded that of the former. For instance, in experiment 30 with Little Club, on the basis of a 14-hour day and a 10-hour night, the 1-day plants had received a total of approximately 150 hours of darkness from the time of their first transfer to the dark chamber until they were photographed (Fig. 11, A), the 2-day plants had received approximately 175 hours, and the plants exposed to light 4 hours daily had received over 250 hours of darkness. Yet the 1-day and 2-day plants bore hypersensitive tissue, while those exposed to light 4 hours daily not only bore no hypersensitive tissue (Fig. 11, B) but supported a rust development indistinguishable in any respect from that of control plants under natural conditions of illumination. The longest continuous period of darkness to which these latter plants were subjected, however, was 20 hours, while the 1-day plants had one period of continuous darkness extending over 2 nights and 1 day, or approximately 36 hours, and the 2-day plants (bearing more hypersensitive tissue) had one period of approximately 60 hours in the dark.

In the face of these facts we can only conclude that the appearance of hypersensitive tissue was associated with some physiological condition of the host protoplasm not attained during recurrent 20-hour periods of darkness but attained by a few cells after a period of 36 hours in the dark and by a much larger proportion of the leaf tissue after 60 hours in the dark. The absence of any correlation between the sum total of time in the dark and the appearance of hypersensitive tissue argues strongly against the possibility that cessation of carbon assimilation was alone responsible for the disturbance of compatible relations between host and fungus.

The cessation of carbon assimilation cannot, of course, be without its effect, for photosynthesis ordinarily provides a substrate for metabolic processes that are continued in the absence of light, and, in so far as low concentration of substrate for these reactions contributes to the altered course of metabolism, referred to here as dark metabolism, to that extent must the cessation of photosynthesis be held responsible for the changes in the relations of the host toward the rust. Dark metabolism, however, if allowed to proceed for a sufficiently long time, comes to involve physiological states that do not occur in the same host under otherwise similar conditions if it is permitted to assimilate periodically. The evidence from the experiments described above indicates that one (or possibly more) of these states, rather than the mere absence of the processes of carbon assimilation, is responsible for a change on the part of the host from congeniality to uncongeniality toward the rust form involved.

III. EXPERIMENTS ON DETACHED LEAVES WITH CONTINUOUS PERIODS OF DARKNESS

A. Conditions of experimentation. Seedlings were grown and inoculated as usual and the rust was allowed to develop until infection fleeks appeared on the leaves. Then, only when they were ready to be transferred to the dark chamber were infected and noninfected leaves cut off and floated on distilled water in Petri dishes or placed in vials with their bases dipping into water. They were then held in darkness continuously for various periods of time. Two such experiments were carried out simultaneously with experiments on seedlings.

B. Effect of continuous darkness on the appearance of host tissues. Details of the effect on host tissues accompany those on infection type in the following section. The course of visible changes was remarkably different in the leaves detached at the time of transfer to the dark from that in seedlings transferred in pots to the dark. In the first place, the gradient from more rapid change at the tips to slower change at the base, which so constantly appeared in attached leaves, was entirely absent from detached leaves. The tips were often the last portion of the detached leaves to lose their green color, and a greater degree of uniformity throughout the main portion of the leaf existed here than in attached leaves. Rates of change also were different, as can be seen from a comparison of the detailed descrip-

tions of detached leaves and seedlings receiving the same treatment. In experiments 47 and 48, for instance, attached leaves held for 6 days in the dark were only very slightly pale, while detached leaves were almost completely etiolated. While such very striking discrepancies did not appear in experiment 96, a comparison of the descriptions of detached and attached leaves on page 56 will reveal certain very distinct differences.

This sequence of outwardly visible changes in host tissue must be taken as indicating a different metabolic course from that obtaining when whole plants are held in the dark. This indication has already found corroboration in the results of certain experiments carried out by G. H. Duff and myself on the respiratory behavior of attached and detached leaves during long periods of dark, for they disclose some interesting differences between the metabolic courses of the attached and detached leaves.

C. Effect of continuous darkness on the infection type. Differences in the effect of darkness on infection type were found associated with the diverse metabolic courses just referred to. On detached leaves from plants subjected to darkness rust development was faster than on attached leaves and was not accompanied by the development of hypersensitive tissue.

LITTLE CLUB, EXPERIMENTS 47, 48 (not illustrated)—54 pots planted Nov. 6, 1929, 27 pots inoculated Nov. 15 (2- $2\frac{1}{2}$ in. high). To dark chamber Nov. 22.

Detached leaves in Petri dishes (Experiment 48):

Reaction of controls—'4+''. Pustules orange instead of reddish brown. (This color difference was associated with high atmospheric moisture content, a relation that has been observed by other investigators).

Effect on infection type of continuous darkness for: 1 day—little effect. Two days—development retarded, final reaction "4+" on slightly pale leaves. Three days—type. "3" pustules in green areas on pale leaves (on 18th day after inoculation). Four to 9 days—gradation from pale green to white to brown leaves, as time in dark increased, all with green areas surrounding rust pustules, which were progressively smaller and paler as the time of continuous darkness increased. On none of the leaves did any hypersensitive tissue appear.

Effect on host tissue of continuous darkness for: 2 days—leaves slightly pale. Three days—leaves pale. Four days—leaf bases nearly white, tips pale green. Five days—bases nearly white, remainder of leaves fairly uniformly pale. Six days—almost completely etiolated. Seven to 9 days—completely etiolated, partially injected, 1 leaf brownish.

Seedlings in pots (Experiment 47):

Reaction of controls-"4+".

Effect on infection type of continuous darkness for: 6 days—"x", necrotic flecks closely resembling Khapli type accompanying some pustules. Teliospores in some small pustules.

Effect on host tissue of continuous darkness for: 6 days-very slightly pale.

LITTLE CLUB, EXPERIMENT 96 (not illustrated)—30 pots planted Oct. 28, 1930, 20 pots inoculated Nov. 6, by spreading spores. To dark chamber Nov. 14.

Detached leaves with cut ends in water:

Reaction of controls-"4".

Effect on infection type of continuous darkness for: 1 day—unchanged. Two to 5 days—(detailed notes kept only up to 2 weeks from inoculation). Pustules somewhat smaller, but even on 5-day leaves rust development kept pace with that on 1-day attached leaves. Pustules surrounded by green areas on progressively paler leaves. No hypersensitive tissue at any time.

Effect on host tissue of continuous darkness for: 1, 2 days—no change. Three days—slightly pale, except the tips. Four days—tips very slightly pale, bases quite pale. Five days—leaves pale, paler at the base than at the tip, none flaccid or with dead areas, but paler than the corresponding leaves of the potted plants.

Seedlings in pots:

Reaction of controls-"4".

Effect on infection type of continuous darkness for: 1 to 5 days—(detailed notes kept until 2 weeks from inoculation). Rust development much slower than on detached leaves receiving corresponding treatment. Hypersensitive flecks developed on plants 3 days or longer in dark.

Effect on host tissue of continuous darkness for: 1 day—no effect. Two days—upper portions slightly pale. Three days—slightly pale with tips pale. Four days—upper quarter of most leaves pale, slightly pale below this, several tips dead. Five days—upper halves of leaves very pale, most leaves not fully turgid, several tips dead, a few leaves with the upper quarter dead.

Recapitulation. It is evident that detached leaves kept in the dark present an entirely different picture from that offered by whole plants in pots. This divergence is expressed in a different sequence of changes in appearance as well as in differences in respiratory behavior. Associated with these indices of a modified dark metabolism following detachment was a difference in rust behavior, the essential feature of which was the elimination of any development of hypersensitive tissue after prolonged periods of darkness. Such relations should prove significant ultimately in directing our search for those physiological processes responsible for the causation of compatibility or incompatibility of the host with a physiologic form of rust.

GENERAL DISCUSSION

In interpreting the experimental results described in this paper, the possible importance of the catabolic phases of host metabolism has been given an emphasis that has not previously been accorded to it in considerations of the effect upon rust infection of modifying host metabolism by darkness. As far as concerns abundance and rate of growth of the rust, there is nothing in the results presented to indicate dependence of the rust on products of synthesis rather than of catabolism, and, as far as concerns the determination of compatible or incompatible relations between host and fungus, the evidence all points to a direct relation not with those processes

dependent on light but with some state reached by those mechanisms remaining active when light is excluded.

My observations do not find an acceptable explanation in the theory hitherto advanced of the effect of darkness upon rust growth, wherein it has been suggested that because of the lack of some substance formed by the host only in the light rust growth ceases in darkness but is promptly resumed when photosynthesis again begins. Such a theory took origin in the equivalence discovered by Fromme (8) and Mains (19) to exist under certain circumstances between the time of continuous sojourn of infected plants in darkness and the time of retardation occasioned in the appearance of rust pustules. But, it is evident from the results here presented that such a relation is not a general one. The tendency was, instead, under the conditions of my experiments, for the period of retardation in pustule development to exceed that of subjection to darkness. This relationship suggests dependence not merely on the cessation in the dark of some process recommenced on return to light but rather on the modification of a physiological state during darkness that was reestablished on return of the plants to light. Furthermore, it frequently happened that rust pustules actually formed and broke through the epidermis of leaves while they were still in the dark chamber. The fungus was, therefore, developing under the influence of the characteristic metabolic sequence of the tissues in darkness, but it developed more slowly than in light and with very different results to the host tissues. Final proof that rust development was not simply arrested in the dark, proceeding in its usual fashion on return to light, exists in the appearance of the insignia of incompatibility following the subjection of congenial host plants to sufficient periods of continuous darkness.

The change from compatibility to incompatibility of host tissues to the rust form employed was not related to the absolute duration of darkness, as it should have been had it been solely dependent on lack of light and, therefore, on cessation of the processes to which light is essential. It was related rather to a duration of darkness, which showed an inverse relation to temperature, and was correlated with the rapidity of metabolic change in the tissues in the dark, as judged by the time required for the progression of dark metabolism to the point of death of the tissues. Under such circumstances it must be the processes remaining active in the dark, not those extinguished by the absence of light, that are important to the observed change in host-fungus relationships.

This is further substantiated by the fact that the change from compatibility to incompatibility was not related to the total dark experience of the seedlings. Interruption of darkness by short intervals of light once in 24 hours prevented the occurrence of well-defined hypersensitive tissue, while comparable seedlings, simultaneously subjected to a shorter total dark

experience so arranged that they received one long period of continuous darkness, bore sharply hypersensitive tissue.

The evidence as a whole can be very well rationalized on the assumption that disturbance of compatibility between host and fungus, as expressed in the appearance of the symptoms of resistance on hosts susceptible to the rust form involved, was consequent upon the establishment of some definite metabolic state in the host cells resulting from subjection of the seedlings to prolonged darkness under the conditions prevailing during these experiments. It is not intended to discuss in physiological terms the possible nature of the metabolic state involved until such time as the results of the researches into its nature now in progress are communicated. For a general conception of the physiological background of these investigations the reader has already been referred to literature on the dark metabolism of various plant organs (1–7), (12), (14), (18), (22), (23), (27).

In terms of this hypothesis, it is assumed that when wheat seedlings of varieties congenial to infection by *Puccinia graminis tritici* p.f.21 were subjected to darkness, they entered upon a definite course of metabolic change, which, if allowed to proceed long enough, led them to a physiological state uncongenial to rust development. The visible result was the appearance of hypersensitive tissue in the presence of fungus mycelium together with the retardation and inhibition of rust development.

After the return of seedlings to light, unless either the host tissues or the fungus mycelium had become irreversibly affected, the fungus was able to develop secondarily in the manner characteristic of the rust form in a congenial host. This may be assumed to be associated with a redirection of metabolism such as is known to occur on the return to light of certain green plant tissues after a period of darkness insufficiently prolonged to bring about a state of irreversibility (1). Once the right metabolic state had been reached, however, hypersensitive tissue developed, even though the plant was returned to light, and the secondary pustules developed under the influence of the redirected metabolism appeared not in the immediate center of primary infection but in the adjacent tissue. If, on the other hand, dark metabolism was not allowed to progress to the hypothetical initial point for production of hypersensitive tissue, but was interrupted at an early stage by a period of light sufficient to restore the original metabolic condition, repeated subjection to such short periods of darkness did not cause a serious disadjustment of compatible relations, so long as the plants were not held continuously so long in the dark that the critical metabolic state, resulting in incompatibility, was reached. This would explain the fact that in one experiment infected plants suffered nearly 300 hours of darkness, so allotted that 4 hours of light always succeeded 20 hours of darkness, without showing symptoms of uncongeniality to rust development or any indications of retardation of rust growth; whereas, on corresponding plants receiving less than 200 hours of darkness but with a continuous period of about 60 hours, a resistant infection type resulted. Shorter daily exposure to light than 4 hours eliminated hypersensitiveness but did not maintain quite the same metabolic conditions as did the longer light periods. The fungus was retarded in its development and was unable to form pustules of normal size, and the host tissues eventually became pale and flaccid and finally succumbed. The failure of hypersensitive tissue to appear under these circumstances indicates a failure of the tissues to reach the metabolic state requisite for the replacement of compatible by incompatible relations and provides additional evidence on which to base investigations into the nature of this hypothetical metabolic state.

In different experiments, where plants differing sometimes in physiological state were also subjected to darkness at different temperatures, the rapidity of progression of the seedlings through their succession of visible changes was affected, and this is taken as an indication of similar effects on the rapidity of metabolic change. The strongly suggested correlation between this rapidity of change and the duration of darkness necessary under any set of conditions for the production of hypersensitive tissue again implies that the disadjustment of compatible relations between rust and host tissues was dependent on the establishment of some metabolic state that appeared in the course of dark metabolism. The slight differences in the duration of darkness required to bring about a change from compatibility to incompatibility, observed in seedlings differing slightly in age, could well be assigned to divergences in rapidity of metabolic change in the dark, resulting from differences in physiological state of the seedlings at the time of discontinuing illumination. That the time relations and the course of dark metabolism may be influenced by the physiological state of the tissues at its initiation is no new idea to students of dark metabolism. Blackman (2), Blackman and Parija (3), Parija (22), and Godwin and Bishop (12) have called attention to the importance of this consideration.

The experiments on detached leaves support the metabolic hypothesis. The course of visible change in darkened detached leaves, distinct from that of attached leaves, indicates a different course of metabolism in these leaves. This view is corroborated by comparative experiments on the respiration of attached and detached leaves, which revealed the fact that dark metabolism in the two differs in certain important respects. The metabolic course in detached leaves failed to establish the condition essential to the appearance of hypersensitive tissue in infected areas and allowed better and more rapid development of uredinia than occurred on corresponding leaves attached to their plants. This is seen as an important guide in a search for

the metabolic state assumed to be responsible for loss of congeniality toward the rust.

The nature of the critical metabolic state cannot be indicated as a result of the experiments described. It might involve measurable concentrations of metabolites, or protoplasmic states, or it might involve both. But the experiments reported here suggest very strongly that the physiological processes of the host that are not discontinued when light is excluded are concerned in the determination of the congeniality or uncongeniality of a host to a rust pathogen. In thus giving prominence to the processes of dark metabolism, they open up a new field for investigation into the nature of the rôle played by host physiology in the determination of rust-infection types.

These researches are being carried on in the Department of Botany, at the University of Toronto, under the direction of Dr. D. L. Bailey and Dr. G. H. Duff, to whom I am indebted for constant interest in the investigation and for invaluable criticism and advice, both in the design of experimentation and in the preparation of manuscript. The investigation has been carried on during tenure of a fellowship of the Ontario Research Foundation and research and teaching assistantships in the Department of Botany. I wish to acknowledge my indebtedness to Dr. M. Newton, of the Dominion Rust Research Laboratory at Winnipeg, for seed of the differential wheat varieties and for a culture of *Puccinia graminis tritici* p.f.21.

SUMMARY

1. Subjection of seedlings of 11 wheat varieties to darkness for periods of from 1 to 7 days resulted in changes in the appearance of the leaf tissues; in the infection type produced by *Puccinia graminis tritici* p.f.21; in the rate of development of the rust; and, in some instances, especially with resistant hosts, in the number of pustules and visible infection centers.

2. Rust development was retarded by darkness, but the retardation was not generally equivalent to the period of darkness. The period of retardation was not generally equivalent to the period of darkness.

tion most frequently exceeded the period of darkness.

3. The modification of infection type on seedlings subjected to prolonged periods of darkness was toward a more resistant type. It involved diminution in size of pustules or elimination of pustules, and appearance of sharply hypersensitive tisssue on ordinarily congenial hosts.

4. Interruption of darkness by short daily periods of light prevented appearance of the sharply hypersensitive tissue resulting from subjection

to continuous darkness.

5. Detachment of leaves at the time of subjection to continuous darkness eliminated the sharply hypersensitive tissue resulting from subjection of infected seedlings to darkness. The effects on the general appearance of

leaf tissues were also different from those on similarly treated attached leaves.

- 6. Evidence was discovered of a correlation between durations of continuous darkness, required under various circumstances to cause appearance of hypersensitive tissue on seedlings, and rapidity of metabolic change in host tissue in the dark, as judged by the duration of darkness required under any set of circumstances to affect the tissue irreversibly.
- 7. On tissues of susceptible seedlings not irreversibly affected by darkening, secondary pustules of a susceptible type appeared in tissue adjacent to resistant primary infections resulting from darkening.
- 8. An hypothesis has been developed concerning the relation of modification of the infection type to the dark metabolism of the host, which offers an explanation of the results obtained and forms a basis for further experimentation.

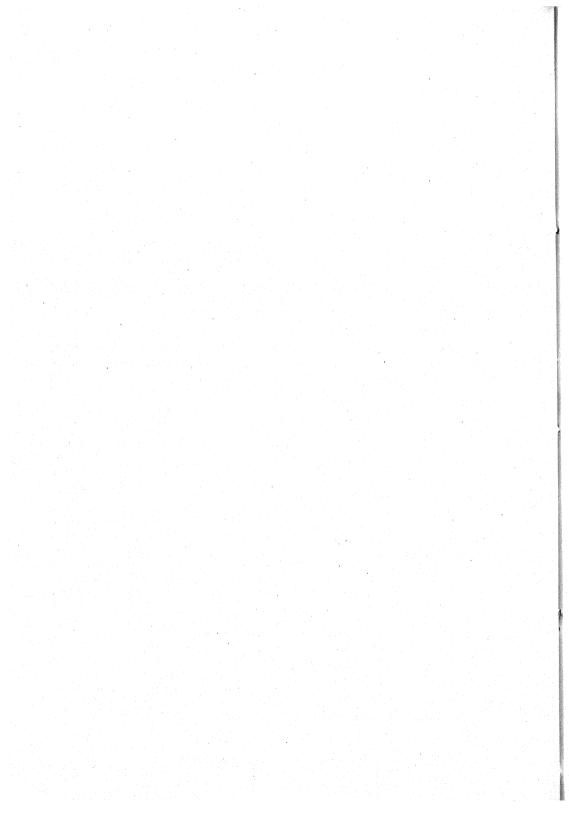
University of Toronto, Toronto, Ont., Canada.

N. B. Approximately one-third of the cost of printing this contribution was borne by the author.

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A ROT OF APPLE FRUIT CAUSED BY PHYTOMONAS MELOPHTHORA, N. SP., FOLLOWING INVA-SION BY THE APPLE MAGGOT¹

T. C. ALLEN AND A. J. RIKER

INTRODUCTION

Bacteria, producing rot of apple, have been isolated during the course of a study on the apple maggot, Rhagoletis pomonella Walsh, and an associated decay. These bacteria were first obtained by the writers from decaying apple tissue infested with the larval or "railroad-worm" stage of the apple maggot. Further isolations of these bacteria were obtained from other decaying maggot-infested fruits, from maggots, and from adult insects. All these strains appeared to possess similar cultural characteristics. Inoculations with them upon apple tissue showed them to be pathogenic. Since the losses due to this rot are quite serious to fruit growers in the Kickapoo-Valley section of Wisconsin and since so little information on this decay or its causal organism could be found in literature, further study of this problem was deemed advisable.

ECONOMIC IMPORTANCE

The economic importance of the rot in maggot-infested apples under some conditions is considerable. Harvey (6) notes, "As the larvae grow and the fruit matures, the enlarged channels do not heal, but turn brown and the presence of the maggots is then readily detected. These channels meander through the whole fruit even to the core. They often cross each other, enlarge as the larvae grow, and in last stages of Trypeta work, run together producing large cavities. Finally they involve the whole fruit.

... "Porter (11) illustrates the injury caused by rot within maggot-infested fruit: "Everyone living in the country, in New England and near-by states, is familiar with the disappointment experienced on biting into a 'railroaded' or maggoty apple. Many such apples give no external warning that anything is wrong, although their interior may be a brokendown mass of rotten pulp." Further mention is made of damage to apple tissue "... caused by the maggots, which, in feeding and moving through the fruit, break down the pulp, leaving brown trails of rotten tissue."

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The writers wish to express their appreciation to Professors Baldwin and Frost for laboratory facilities and advice and to Professor Wilson and to Professor Granovsky for the inception of the problem and for suggestions during the earlier stages of the work.

The insidious destruction following the invasion of this maggot was emphasized by Chapman (4): "I feel rather certain that growers have been in a large number of cases unaware of the severity of the pest in their plantings. Winter fruit especially may appear perfectly sound externally at harvest to one unfamiliar with egg punctures, made earlier by the apple maggot fly, only to break down and become worthless some weeks later when it comes into the possession (often several persons removed) of the purchaser."

Decomposition in a 60-bushel lot of maggot-infested Dudley apples, held in the packing house about a week after picking, was witnessed by the senior writer during the summer of 1930. At the end of 10 days the damage due to decay and collapse of the fruit about conspicuous brown trails in the apples was so great that re-sorting was necessary. Twenty bushels of this lot were found worthless. The remaining 40 bushels appeared healthy and were therefore kept for sale to local consumers. An inspection of these apples after another week, however, showed that many had developed symptoms similar to those encountered before. Twenty-two of these 40 bushels were sorted out as healthy during a second overhauling. Later, all of them were lost because of tunneling of maggots and apple decay.

ASSOCIATION OF THE APPLE MAGGOT WITH DECAY

The association of the apple maggot with a decay in the apple has been frequently observed by those familiar with this insect. Riley (12) associates a rot in apples infested with this magget that, ". . . burrows in all directions, and in varied numbers, in the flesh of the fruit, giving it a discolored, honey-combed, rotten, and filthy appearance." Illingworth (7) has noted in certain maggot-infested apples, ". . . a black rot that leaves the fruit a dry, hardened mass." Porter (11) wrote: "If several maggots are present in one apple the whole interior finally breaks down, and the apple becomes a worthless mass of rotten pulp, although it may still appear perfect externally. This injury is very characteristic, and there is little danger of confusing it with that caused by any other insect." Patch (9) reports: "The apple maggot works in soft discolered mushy trails anywhere in the pulp of the apple." Pettit (10) observes that "The apple maggot bores indiscriminately through the flesh, piercing a tunnel which is of small bore for the greater part of its length, although toward the last the tunnel may, and often does, develop into large blackened cavities, excavated near or in the core." Granovsky (5) states: "Many infested apples give no external sign, having a quite healthy appearance, although their interior may be a broken-down mass of rotten pulp. The apple maggot in addition carries a bacterial organism which causes and hastens decay of apples in cold storage." A bacterial rot that commonly accompanies the work of the maggot has been found during the past 2 years at Gays Mills, Wisconsin, as already mentioned in a preliminary report (Allen, 1).

The insect in its various stages seems to introduce the rot-producing bacteria into the apple tissue and to hasten its dissemination throughout the fruit.

Two stages of the insect, the adult and the larvae, appear to assist the distribution of these bacteria. The adult or fly seems to provide opportunity for the dissemination and entrance of the bacteria into the host. The entrance of the bacteria into the apple is apparently accomplished with the deposition of the egg beneath the cuticle. That rapid translocation of the bacteria in the fruit as associated with the maggot is shown by the rot of the apple tissue adjacent to the maggot tunnels. This apparent relation between the pathogenic bacteria and the insect suggests biologic transmission of the bacteria similar to that found by Leach (8) in his studies of the potato-black-leg disease. The evidence available on the association between these maggots and the bacteria is considered in detail with the isolation studies of the bacteria.

The purposes of the present paper are primarily to describe the symptoms of this apple rot, to present evidence on the pathogenicity of the causal bacteria, and to give the results of studies on their bacteriological characters.

SYMPTOMS OF DECAY

The symptoms of this disease were similar (1) under natural conditions both in the orchard and in storage and (2) following inoculations under artificial conditions.

In the orchard the rot of the apple tissue may be described from the deposition of the eggs by the insect (Fig. 1, A and C). Maggots that emerged from eggs under the skin in the cheek of the comparatively green apple left minute light brown burrows (Fig. 1, B) that may often be traced from the feeding larvae to the empty eggshell. When such apples fall to the ground, the development of the feeding larvae is greatly hastened and the burrows become larger and darker brown in color, chiefly due to the advancement of decay in the tissue adjacent to their tunnels. The young larvae generally fed and bored their way toward the core. In the course of their development in this area a comparatively firm rot usually appeared about the core of the apple (Fig. 1, E, F, and G). In most cases few or no outward symptoms of this decay were yet apparent. As the apples ripen the dark brown tunnels left by the feeding larvae would often progress to the outer portions and could be seen from the surface of the fruit (Fig. 1, D). Such fruit was commonly found on the ground in infested orchards after apple harvest. The development of the rot in the orchard paralleled in many respects that found in storage.

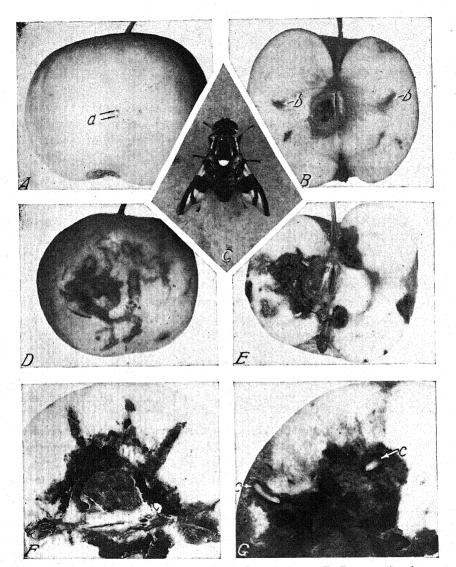


Fig. 1. A. Egg punctures in the surface of an apple, a. B. Burrows, b, of young larvae in apple tissue. C. The adult female of *Rhagoletis pomonella*. $\times 3$. D. Burrows made by apple maggots under the skin of an apple showing early stages of decay. E and F. Section of an apple showing maggot injury and subsequent bacterial decay. G. Later stage than F. Two larvae, c, appear near the margin of the decaying tissue.

In storage, the early symptoms of the disease following the inconspicuous egg puncture and larval burrow occurred as internal decay within and about the core of maggot-infested apples. The line of demarcation between the

healthy outer portion of the fruit was rather sharply delimited from the decayed inner portion. Such symptoms within the fruit have been commonly noted in many early and late varieties of apples. As infested fruit became fully ripened, larvae that were feeding about the core of the apple tunneled to the outer portions of the fruit.

The common later symptoms in storage appeared as brown rot adjacent to insect burrows near the surface of the fruit (Fig. 1, D). Under favorable conditions the centers of decay rapidly advanced from these burrows into the surrounding healthy tissue. Fruits showing these symptoms were easily crushed and uninvaded portions of the healthy tissue could be readily separated from the invaded tissue. Before total destruction of the fruit occurred, the maggots bored their way out of the apples through a conspicuous exit hole. This hole often became surrounded by an area of decaying tissue. Further progress of the rot turned the entire apple into a comparatively firm but shrunken mass of decayed tissue.

Symptoms of the disease produced upon entire apples and apple plugs by inoculation from pure cultures of the bacteria were similar to those found in apples under natural conditions. Inoculations were made on apples surface-sterilized with bichloride of mercury, 1 to 1,000. Checks were made by pricking the surface of the apple opposite the point of inoculation. On entire apples, the first symptom of decay in tissue around the point of inoculation appeared as a small, light brown, slightly sunken area (Fig. 2, A). As this decaying area enlarged, it was preceded by a very narrow water-soaked halo. The rot became darker brown in color as it aged (Fig. 2, B and C). Concentric rings sometimes appeared in the surface of the diseased tissue. The epidermis of the apple seemed comparatively firm, leathery, and slightly sunken. Within the apple, the decay progressed in all directions (Fig. 2, E) from the point of inoculation, and the advancing margin of the rot could be rather sharply distinguished from the healthy tissues. As the fruit ripened it seemed to become more favorable for the growth of the bacteria, for the decay was more rapid. Entirely decayed fruit was irregular in shape, shrunken, and dark brown (Fig. 2, D). The tissue remained rather firm.

Plugs for inoculation studies were made from fresh apple tissue, with aseptic precautions, in the usual manner. These plugs varied in size and shape, but were generally $\frac{1}{2}$ to $\frac{3}{4}$ in. square and $\frac{1}{4}$ in. thick. Cylindrical plugs also were obtained by using a sterilized $\frac{5}{8}$ in. cork borer. The freshly cut tissue was immediately placed on agar previously plated in Petri dishes, inoculated, and then incubated at a temperature between 18 to 22° C.

The first symptom of the disease following inoculation of apple plugs, was usually a small brown circular region about the needle prick. This invaded portion became darker, with a narrow water-soaked margin, and

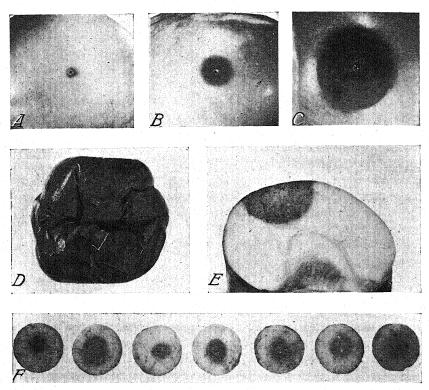


Fig. 2. A. Rot produced 2 days after inoculation about a needle puncture in a Pearmain apple. B. As A, except 4 days after inoculation. C. As A, except 16 days after inoculation. D. Entirely decayed apple several weeks after inoculation. E. Section of an apple showing decay 16 days after inoculation. F. Discs made from apple tissue showing decay about needle punctures 2 days after inoculation with the 7 single-cell cultures of bacteria from left to right, respectively.

advanced from the point of inoculation as the organism invaded adjacent tissue. A conspicuous brown area was produced by the bacteria in 24 hours. In early stages of rot, the apple tissue remained moist and often a surface coze containing great numbers of bacteria appeared about the needle prick. Several days were generally required for the entire plug to decay. At this time the organism had penetrated the apple tissue and formed a colony on the agar. Plugs completely rotted appeared brown and later dried down. Separate plugs in each Petri dish were used as checks by pricking them with sterile needles.

ISOLATION OF BACTERIA

Seven strains of bacteria were isolated from maggot-infested apple tissue and from different stages of the insect. The bacterial strains under consideration were obtained during the fall of 1929 and during 1930 from different varieties of apples and from 2 localities in Wisconsin. Geographically, strains Nos. 1, 2, 3, and 4 were secured from Gays Mills, Wisconsin, and strains 5, 6, and 7 from Fort Atkinson, Wisconsin.

Strain No. 1 was secured from isolations made from young insect larvae transferred from beginning stages of rot in a Wealthy apple.

Strain No. 2 was secured from isolations made from advanced margins of decay about larval burrows in Snow apples.

Strain No. 3 was secured from the surface of an adult apple-maggot fly caught in the orchard.

Strain No. 4 was secured from the dissected abdomen of a female adult apple-maggot fly. The abdomen of the fly was seared before making an incision and a portion of the alimentary canal was withdrawn and placed on culture media.

Strain No. 5 was secured from larval burrows in a Greening apple that was in the early stage of decay.

Strain No. 6 was secured from larval burrows in an unnamed sweet apple.

Strain No. 7 was secured from the surface of a young larva that was producing brown trails of decayed tissue in a Greening apple.

Isolations of the organism from invaded apple tissue were accomplished with the usual laboratory technique. Before such isolations were made the apples were surface-sterilized with bichloride of mercury 1 to 1,000 and the fruit cut or broken open with a sterile scalpel. In isolating the bacteria from the apple proper, portions of the recently invaded tissue were transferred either to water blanks or melted agar, macerated, and used in dilution plates of nutrient glucose agar. In isolating the bacteria from the surfaces of the larvae, the maggots were removed from the diseased tissue and placed on agar in Petri dishes. Individual colonies of the bacterium were usually obtained from the plates following a period of 2 or 3 days. Approximately 20° C. was a satisfactory incubation temperature.

Numerous yeasts as well as bacteria appeared on the poured plates. Attempts to produce rot by inoculations into apple with the yeasts alone all gave negative results. To discourage these yeasts, crystal violet (1 to 200,000) was added in some series to the nutrient glucose agar.

The bacterial cultures were purified as described later.

PATHOGENICITY

The pathogenicity of the bacteria under consideration was determined by successive isolations and inoculations. Two series of inoculations were made with each of the 7 purified strains of bacteria on Wealthy apple tissue. Each strain was inoculated in duplicate into apple plugs and also in duplicate into entire apples. Unless otherwise stated, the inoculated apple tissue was kept at room temperature. The plugs from Wealthy apples were prepared under aseptic conditions and placed in Petri dishes on 10 cc. of agar intended to maintain the moisture. Three plugs were distributed in each of 7 Petri dishes with the usual precautions to prevent contamination. A strain of the bacteria was inoculated, respectively, with a needle into 2 of the plugs in a dish. The remaining noninoculated plug was pricked by a sterile needle and used as a check. The water-soaked halo appeared in about 12 hours. In every case the rot was conspicuous in 2 days (Fig. 2, F) and usually involved the whole plug in a week. No rot developed in the controls.

Reisolations of the different strains of apple-rot bacteria were secured from the above series of inoculations made into apple plugs. Small sections were taken from the most advanced portion of decaying tissue and macerated in Petri dishes. Subsequently dilutions were made and colonies of the bacteria appeared in the poured plates that resemble the original cultures of the apple-rot organism.

Reinoculations of the different strains of bacteria secured from these reisolations were made into apple plugs. The technique employed in this work was identical to that already described in the first series of inoculations of the bacteria on apple plugs. Rot was produced after 48 hours about the needle pricks of the reinoculated cultures. No rot developed in the control plugs.

Entire Wealthy apples also were used to test the pathogenicity of each of the 7 bacterial strains. Before inoculations were made the apples were washed and surface-sterilized with bichloride of mercury, 1 to 1,000. One apple was used for each strain. The apples, respectively, were then inoculated twice with each strain of the bacteria. Two sterile needle punctures were made as controls. The apples were placed under sterilized inverted beakers at room temperature. The first symptoms became apparent about the inoculations within 2 days (Fig. 2, A). After this time the rot became progressively more and more conspicuous (Fig. 2, B, C, D, and E). No rot developed about the control punctures.

Reisolations of the different strains of apple-rot organism were secured from the above series of inoculations made into entire apples. The epidermis of the apple above the most advanced area of decay was removed and a small portion of the rotted tissue was transferred to Petri dishes and macerated. Subsequent dilutions and poured plates were then made. Colonies that resembled the original cultures appeared in 48 hours in the agar of the poured plates.

Reinoculations of the different strains of bacteria obtained from these reisolations were made into entire apples. The technique employed in this

work was a repetition of that already described in the first series of inoculations of the bacteria on entire apples. Rot was produced in a few days about the needle pricks of the reinoculated cultures.

Additional inoculations were made either into entire apples or apple plugs made from Delicious, Duchess, Dudley, McMahon, McIntosh, Patten's Greening, Rome, Snow, and an early sweet russet apple. Rot was produced in all the trials made with these apples.

Susceptibility of different varieties of apples or related fruits to invasion by this bacterial organism has not been studied. It was noted, however, that in some apples many weeks were required for the organism to rot the entire fruit. This was observed in more firm varieties, as Northwestern Greening, and in comparatively mellow varieties, such as McMahon. On the other hand, such varieties as the early sweet russet, Delicious, Wealthy, and Dudley, which are sweet and mellow, decayed more readily. These limited observations appear in correlation with Porter's (11) statement that the apple maggot seems to be attracted to sweet apples in which the flesh is more easily broken down by the larvae.

Green apples appeared less susceptible to rot than ripe apples in experiments on Wealthy, McIntosh, and Northwestern Greening. Two apples of each variety were inoculated through needle punctures in 3 places of each apple. Equally numerous control punctures were made. Little or no rot followed these inoculations. These apples were picked from the trees 2 weeks following the inoculations and held in storage for observations, but no rot developed. Parallel inoculations and control punctures were made into apples of the same varieties a few days after they had been picked. The inoculated fruits generally developed considerable decay within a suitable incubation period.

BACTERIOLOGICAL STUDIES

A favorable medium on which to grow the bacteria during the isolation and inoculation studies was sought in preliminary studies. Tests on different media showed very little growth by the bacteria in beef-extract and Bacto-peptone broths unless a sugar were present. As described later in this paper, the most vegetative growth was made in media containing glucose. Nutrient glucose agar was found satisfactory for this isolation work.

Separation of the 7 different bacterial strains previously mentioned from yeasts and other organisms was tried by several successive replatings with the usual technique. The medium employed was nutrient glucose agar. The dilution plates were incubated at room temperature for a period of 2 or more days. Individual colonies were picked from dilution plates in which fewer than 60 individual colonies appeared per plate. Additional

series of plates were poured if the growth suggested the presence of an undesired organism in the culture.

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The technique employed in the bacteriological studies during the fall of 1930, unless otherwise noted, was that given by the Committee of Bacteriological Technique of the Society of American Bacteriologists (13). Each of the tests was run in duplicate with suitable controls. These duplicate tests were made in most cases 3 different times for each of the 7 strains of bacteria under consideration. All the determinations for an individual character were similar, except as noted, for each of the 7 different strains. Unless otherwise mentioned, the cultures were incubated at approximately 21° C.

MORPHOLOGICAL STUDIES

The shape and size of the bacteria were determined from slides made from 18-hour-old cultures on nutrient glucose agar and stained with Gram's stain. The vegetative cells observed were short rods, with round ends arranged singly or in pairs, and at times in short chains. A hundred measurements with a filar micrometer showed that the bacteria were 0.6 to 1.0 by 0.8 to 1.9 μ in size, with the greatest number about 1.32 by 0.68 μ .

Motility was observed shortly after transfers began to grow. After the cultures were from 18 to 24 hours old, however, their movement diminished. Motility was demonstrated in hanging-drop cultures and in suspensions mixed with India ink.

Flagella stains showed that the bacteria possessed lophotricheous flagella. Strains Nos. 1 and 2 only were used for staining flagella. With Casares-Gils' flagella stains the writers were able to observed 2 polar flagella. Mr. H. E. Sagen, however, demonstrated still more clearly the presence of 2 polar flaglla with Loeffler's, Gray's, Casares-Gils', and Bailey's (2) method of staining flagella.

Young cultures of the apple-rot bacterium were Gram-negative, but with age Gram-positive cells appeared. Considerable variation of the bacteria to Gram's stain was at first noted in 2-day- to 6-day-old cultures. As a check on the technique (Stitt, 14), the apple-rot organisms were always stained on slides between smears of Gram-positive and Gram-negative organisms. Cultures of the following ages were employed: 12, 16, 20, 24, 28, and 36 hours, 2, 4, 8, 16, and 32 days. It was found that, while all strains of the apple-rot organisms less than 24 hours old gave consistent Gram-negative results, distinct Gram-positive organisms appeared to increase in number in the older cultures.

Capsules have not been demonstrated. Efforts were made to find them by examining suspensions of 18-hour-old cultures in India ink and by staining with Hiss's and with Gin's capsule stain. India-ink preparations were made on slides by adding a loop of India ink to a suspension of motile bacteria.

Tests for the formation of spores were negative. Thirty-day-old cultures were stained according to the Dorner and Ziehl-Neelsen methods, but no spores were detected. Thirty-day-old cultures, grown on nutrient agar, also were heated 12 minutes at 85° C. and transferred to nutrient glucose agar. No growth appeared.

GROWTH CHARACTERS ON VARIOUS MEDIA

The colony characters were determined on nutrient agar containing 2 per cent of glucose. An incubation temperature of about 20° C. was maintained for all these studies. The bacterial colonies appeared on the medium after 36 hours as minute circular, almost transparent, growths. After 3 days they appeared as follows: Surface type: growth, rapid; form, circular; surface, smooth, glistening; elevation, convex; edge, smooth, entire; internal structure, finely granular; optical characters, opaque; chromogenesis, light pink. This color usually appeared in 4 to 7 days. The intensity of color and the time at which this chromogenic character appeared varied considerably. In cultures several weeks old, as the agar dried, the pink tint became somewhat brown. This chromogenic character was of considerable assistance during the earlier isolation and purification studies. It appeared, however, not always to be a constant character on different media.

Deep type: Form, lens-shape; internal structure, dense. Deeply submerged colonies made very little growth.

Agar strokes, incubated 4 days at 18° C. on nutrient glucose medium, had these characters: growth, moderate; form of growth, contoured to filiform; elevation, raised; surface, smooth; optical character, opaque; chromogenesis, usually pink; odor, absent; consistency, butyrous; medium, unchanged in color.

In gelatin stabs no liquefaction occurred and the medium was unchanged in color. A very scanty villous form of growth was generally made along the line of puncture.

In nutrient broth, with 2 per cent glucose, good growth of the organism was made in 24 hours. The medium was slightly cloudy. Sediment was formed, which was viscid on agitation. In 48 hours the amount of sediment was abundant and compact. A pellicle was formed. The broth became slightly clouded by a flocculent development that, on agitation, dispersed as a fine granular suspension.

Sugar-fermentation studies were made in nutrient broth. The organism was allowed to grow 5 days in 1 per cent glucose, saccharose, and lactose broths containing Durham tubes. Tests of the hydrogen-ion content by the colorimetric method were then made. Acid was formed from glucose and

sucrose, but very little, if any, was found in the medium containing lactose. Gas was not formed in any of the fermentation tubes. The density of pellicle and sediment showed that the growth made was more pronounced in the medium containing 1 per cent glucose. The least amount of growth was made in 1 per cent lactose.

The diastatic action of the bacteria was determined on 2 per cent starch agar. Starch was not hydrolyzed. Little growth occurred after the first 24 hours following the transfers. After an incubation period of 7 days at room temperature a saturated solution of iodine in 50 per cent alcohol was poured over the agar. No clear zone developed about streaks in any of the plates.

Reactions in milk were recorded from studies of inoculated litmus-milk cultures. Such cultures were incubated at various temperatures and were observed over a period of 6 to 8 weeks. The litmus milk showed little, if any, change.

Reduction of nitrates was tested in the usual way. Tubes of $\frac{1}{10}$ per cent of potassium nitrate broth, containing Durham tubes, were inoculated with the bacteria. The broth contained no sugar. In 7 days only a slight growth was made by the organism, which appeared as a scanty, white, finely granular sediment on the bottom of all test-tubes. No gas was formed. Tests with Trommsdorf's reagent showed that no nitrites were present. Trials with diphenylamine showed that the nitrates were still present.

No indol was found in tests made with 6-day-old cultures. A scanty, white, chalky sediment had settled on the bottom of these tubes, which indicated that only little growth had occurred. Indol tests were made according to Ehrlich-Böhme's technique.

STUDIES OF CULTURES DERIVED FROM INDIVIDUAL CELLS

Individual cells were picked and grown from each of the 7 apple-rot organisms under consideration, so as to remove, as far as feasible, the possibility of the presence of some undesired organism. Although the writers had as much confidence in the cultures secured by repetitions of the poured-plate method as this technique justifies, the published (15) and unpublished results secured at Wisconsin by this method from supposedly pure cultures made the writers wish to check some of the earlier results with the apple-rot bacteria against cultures grown from individual cells. A number of single-cell cultures were secured through courtesy of A. A. Hendrickson, which were isolated with the double Chamber's micromanipulator and grown as recently described (15). Two single-cell cultures from each of the 7 strains under consideration were chosen for further study. They were used in a number of selected bacteriological determinations, as described in the following pages. Unless otherwise stated, all these bacteriological determinations were made in triplicate. Pathogenicity tests were

made with the single-cell cultures, on entire Delicious apples. Rot was produced by all the cultures following inoculations into entire apples, as previously described in the pathogenicity studies.

The same bacteriological characters were found for the 14 single-cell cultures as for the 7 original strains in regard to Gram's stain, nonliquefaction of gelatin, nonhydrolysis of starch, no change in litmus milk, no reduction of nitrates, and no production of indol.

Some additional determinations were made with these single-cell cultures.

The rate of growth at various temperatures was determined by measuring the diameter of bacterial colonies grown on nutrient glucose agar. Two strains of the single-cell bacteria were used, so as to make a total of 36 colonies at each temperature. Only 6 colonies were grown in a Petri dish that contained 20 cc. of agar. The loss of moisture from the agar was mostly prevented by sealing the plates with adhesive tape. The plates were incubated at each of the following temperatures: 4, 8, 13, 17, 21, 25, 28, and 32° C. In 8 days the average diameters in millimeters of the colonies were, respectively, 0.0, 0.5, 1.7, 2.7, 4.6, 5.1, 4.5, and 4.0. In 16 days they were, respectively, 0.8, 1.1, 2.6, 3.8, 7.7, 7.6, 6.6, and 6.4. The results of these studies indicate that the maximum vegetative growth of the bacteria on nutrient glucose agar is made between approximately 21 and 25° C.

Actions of the bacteria on different carbohydrates and related substances were tested in peptone-salt medium, like that employed by Wright et al. (15). Cultures of each strain were run in triplicate in broth containing 0.5 per cent of these substances, which were autoclaved separately and added under aseptic conditions: arabinose, glucose, galactose, sucrose, maltose, lactose, levulose, starch, dextrin, inulin, mannitol, and glycerin. When the cultures had incubated at room temperature for 7 days, the hydrogen-ion concentration was determined by the colorimetric method. The results are recorded in table 1. In accordance with the earlier tests made upon several sugars, the most growth and the largest amount of acid were produced in the glucose broth.

DISCUSSION

The characters of this apple-rot organism do not appear similar to those given in any description that could be found for a known bacterial plant pathogen. The kind of rot produced on apple fruit along with the bacteriological characters seems to distinguish it from any plant-pathogenic bacteria that might be considered as close relatives. Consequently, in consideration of the rot it produces, this apple-rot organism is named, according to Bergey's system (3), Phytomonas melophthora, n. sp. This name was selected in consultation with Professor A. G. Laird. Synonyms according to other systems of classification in common use among plant

TABLE 1.—Hydrogen-ion concentration produced by the various strains of apple-rot bacteria from selected carbohydrates and related substances after an incubation of 7 days in peptone-salt medium at room temperature

Substances tested	Hydrogen-ion concentration from strains number:									
	1	2	3	4	5	6	7	Contro		
Arabinose	4.4	4.4	4.5	4.4	4.4	4.4	4.4	7.0		
Glucose	3.8	3.7	3.9	3.6	3.9	3.9	3.9	6.9		
Galactose	4.2	4.2	4.2	4.2	4.2	4.2	4.2	6.9		
Sucrose	4.4	4.4	4.5	4.4	4.5	4.6	4.6	6.9		
Maltose	6.7	6.7	6.8	6.7	6.7	6.7	6.8	7.0		
Lactose	6.9	6.9	6.9	6.9	6.9	6.9	7.0	7.0		
Levulose	6.3	6.3	6.4	6.3	6.4	6.3	6.3	6.9		
Starch	6.9	6.9	6.9	7.0	7.0	6.9	6.9	6.9		
Dextrin	6.8	6.9	6.8	6.8	6.9	6.9	6.9	6.9		
Inulin	6.9	6.9	6.9	6.9	6.9	6.9	6.9	6.9		
Mannitol	6.5	6.5	6.6	6.5	6.7	6.7	6.7	6.9		
Glycerine	5.9	5.9	5.9	6.0	6.0	6.0	6.0	6.9		

pathologist, are *Pseudomonas melophthora*, n. sp., and *Bacterium melophthorum*, n. sp. A brief characterization follows:

Phytomonas melophthora, n. sp.

This organism was found to be a short rod, 0.8 to $1.9 \,\mu$ by 0.6 to $1.0 \,\mu$, average 1.32 by 0.68 µ, motile by means of 2 polar flagella, Gram-variable and without spores. On nutrient glucose agar incubated at 20° C. for 3 days the colony characters were: growth, rapid; form, circular; surface smooth, glistening; elevation, convex; edge, smooth, entire; internal structure, finely granular; optical characters, opaque; chromogenesis, light pink. Deep colonies were lens-shape and the internal structure was dense. Gelatin was not liquefied. Little growth appeared in standard bouillon broth. Acid, but no gas, was formed from arabinose, glucose, galactose, sucrose, levulose, and glycerin. Traces of acid also were produced from maltose and mannitol. Very little, if any, acid was detected in the media containing lactose, starch, dextrin, or inulin, respectively. Starch was not hydrolyzed. Milk was practically unchanged. Nitrates were not reduced. Indol was not formed. No hydrogen sulphide was given off. The most abundant vegetative growth occurred approximately between 21 and 25° C. The bacteria have been found in association with Rhagoletis pomonella and have been shown to cause decay in comparatively ripe apple fruit.

SUMMARY

1. A bacterial rot of apple fruits has been observed both in the orchard and in storage that appeared to be associated with the apple maggot.

- 2. Bacteria that produced rot in apple fruits have been isolated from the decaying apple tissue and from both the larvae and adults of the apple maggot.
- 3. Seven different isolations of these bacteria from different sources have been purified and followed through the following cycle: inoculation into apples, production of decay, reisolation, comparison with original cultures, and reinoculation into apples followed by the typical rot.
- 4. Bacteriological studies are described. This organism is called *Phytomonas melophthora*, n. sp.

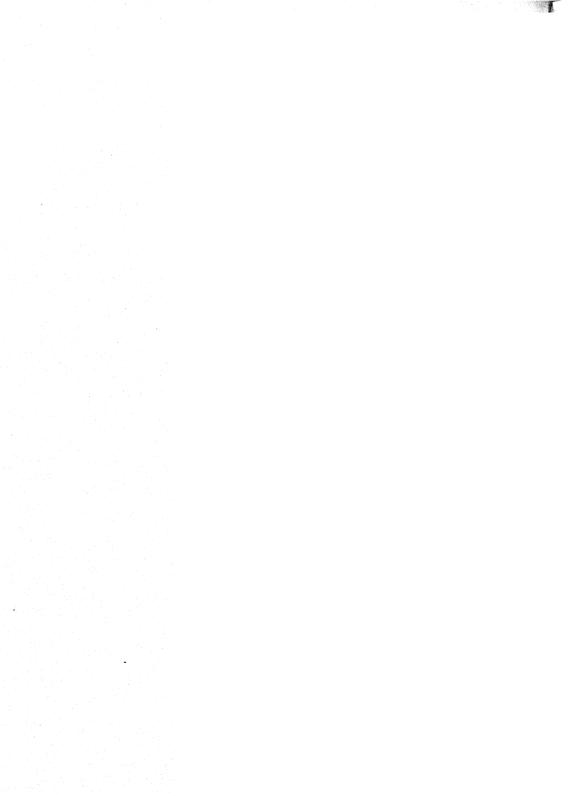
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THE BASIS FOR SCLEROSPORA SORGHI AS A SPECIES

WM. H. WESTON, JR., AND B. N. UPPAL¹

As study of the Sclerosporas progresses it becomes evident that increasing knowledge necessitates changes in the status of certain members of the genus. Of these Scl. graminicola var. Andropogonis sorghi of Kulkarni clearly demands revision.

This fungus was first mentioned by Butler in 1907 (2), when he reported the oogonial phase of a Sclerospora on Andropogon sorghum L. from the Bombay and Madras Presidencies, India, and, impressed by its similarity to that of Scl. graminicola (Sacc.) Schroet. on Pennisetum typhoideum L., tentatively assigned it to that species.

In 1913 Kulkarni (9) found not only the oogonial but also the conidial phase of the fungus on sorghum in the Bombay Presidency. He was impressed by the similarity in the oogonial phase, previously noted by Butler, and by the realization that on these phases specific distinctions are based in some related groups, but he noticed that the conidia germinated invariably by hyphae and never by emission of zoospores as in typical Sclerospora graminicola and decided that this, with other minor distinctions in the effect on the host and in the characteristics of the conidiophores and sterigmata, justified giving the fungus varietal rank as Scl. graminicola var. Andropogonis sorghi.

In 1913 Ito (8, p. 218), discussing the Sclerospora of sugar cane recently described by Miyake in Formosa, suggested separating the genus Sclerospora into Eusclerospora, with conidia germinating by zoospores, and Peronosclerospora, with conidia germinating by hyphae, and included Kulkarni's variety on sorghum in the subgenus Peronosclerospora and thus emphasized its alliance to the truly conidial oriental forms rather than to the zoosporangial type of Scl. graminicola. In 1918 Butler (3, p. 205), in a detailed discussion of the species of Sclerospora known from India, compared typical Scl. graminicola with the variety on sorghum and suggested: "It is not improbable that these differences [in germination and sterigmata] will eventually be considered of sufficient importance to constitute distinct species, but the life history and, in particular, the ability of these fungi to pass from one host to another, is not yet sufficiently known for a decision to be arrived at." In 1924 Weston (14, p. 781), after studying the conidial phase of typical Scl. graminicola on Setaria in the United States and comparing it with the material of the variety on sorghum kindly furnished by Dr. Butler, decided that the variety "is certainly a distinct

1 The writers take this opportunity of thanking Mr. M. K. Desai for the ungrudging assistance rendered throughout this work.

species, one which, aside from other differences, needs only the absolute criterion that its conidia lack an apical papilla of dehiscence to distinguish it without question from S. graminicola. It is clearly a species closely allied to the destructive, predominantly conidial Sclerosporas of the Orient even though it is apparently connected with an oogonial stage, presumably that of S. graminicola." In 1928 the same writer (16, p. 937) again emphasized this point, and since then additional suggestions have been made that Kulkarni's variety is indeed of specific rank.

Moreover, biological evidence also has accumulated to substantiate this view. In 1918 it was first suggested by Butler (3, p. 223) that "In India the bajra (Pennisetum) fungus will not attack jowar [Andropogon sorghum] leaves and vice versa. It is also noticeable that the fungus in some localities in India is confined to one host, even though some of the others may be growing near by. Thus in Pusa it has only been found on Setaria italica though jowar and bajra have been grown in the vicinity. This suggests that the parasite has split into specialized races, each confined to one or more hosts, as in cereal rusts." In recent years the work of Uppal (10) and of Uppal and Desai (11) has given increasing evidence of the correctness of Butler's assumption.

When all these points are considered the time seems favorable for presenting in the following paper the evidence that to the writers seems a convincing basis for establishing *Sclerospora sorghi* as a distinct species.

STRUCTURAL DISTINCTIONS

The life cycle of this fungus on sorghum regularly comprises both a conidial phase and an oosporic phase, as does that of the type species Sclerospora graminicola on Pennisetum and Setaria. The material for study was collected from the leaves of infected sorghum plants at Poona, India, during the period of optimum conidiophore production at night and was carefully compared with similar material of the type species Scl. graminicola on P. typhoideum from the same locality and with the United States material previously studied by Weston (14, 16). The material was also killed and preserved in alcohol or in dilute chromacetic acid and was studied and drawn either in these original preservatives, dilute glycerine, or lactophenol. The preserved material gave smaller measurements of the conidia and conidiophores than the fresh, living material on which all measurements given herein are based.

The conidiophores bear a branch system of usually 3 primary branches approximately equal in size and in extent of development, spreading out from the main axis in close succession so that the conidia borne on the tips of the branchlets lie in a hemispherical plane (Pl. I, B, 1, 2, 4), while in Sclerospora graminicola, as already pointed out by Weston (14), the

branches commonly are not of equal rank since usually one can trace up through the branch system a more or less definite continuation of the main axis, from which short branches grow out abruptly at irregular intervals so that the conidia lie in irregularly disposed bunches (Pl. I, A, 1, 2, 4). This distinction, although not emphasized by Kulkarni (9), is none the less brought out when his plate 7, figures 6 and 7, is compared with his figure 3.

Moreover, the sterigmata (i.e., papillae of Kulkarni), the tapering, ultimate branch tips on which the conidia are borne, tend to be somewhat longer in the sorghum fungus. This was emphasized by Kulkarni as an important distinction, the average lengths being $16.3\,\mu$ for those of the fungus on sorghum as opposed to $8.3\,\mu$ for those of Sclerospora graminicola on Pennisetum. Although more extensive comparisons of a wider range of material show this feature to be more variable than Kulkarni supposed (cf. Pl. I, A and B, 2 and 4), it still remains a difference of corroborative value in distinguishing the two.

Moreover, the conidiophores of the sorghum fungus almost invariably show a transverse septum located usually about halfway between the conidiophore base and the beginning of the branch system, thus cutting off a distinct basal cell below from the main axis proper above (Pl. I, B, 1-4). The septum developing as a thick centripetally closing ring of modified wall substance is usually complete in mature conidiophores (cf. Pl. I, B, 3) and usually single, the presence of more than one being exceptional (Pl. I, B, 9, 10). The basal cell begins somewhat knobbed or swollen and then continues with an approximately constant diameter of 7 to 9 μ to the septum, its length being usually equal to the main axis above (i.e., about 100 μ), occasionally slightly longer (Pl. I, B, 10), rarely distinctly shorter (Pl. I, B, 8).

In Sclerospora graminicola, on the contrary, the base may show some modification (Pl. I, A, 1, 2) in that the wall may be thicker and of slightly different composition compared with the main axis above, but only very rarely is a septum formed delimiting a recognizable basal cell (Pl. I, A, 3) (cf. also rare cases figured by Weston 14, Pl. 2, Fig. x and y; Weston 15, Fig. 4, j, for American material). In this species, also, the basal portion, if thus modified, usually extends less than $\frac{1}{3}$ of the total length of the upright axis, i.e., around 40 μ out of 150 to 200, and usually increases rapidly in diameter from 8 to 10 or 12 μ in this distance.

The conidia themselves, however, present features most sharply distinguishing *Sclerospora sorghi*. In shape, for example, they are most commonly broadly rotund, with the greatest diameter at the middle and the ends equally bluntly rounded (Pl. I, B, 5a, 6, 7a), whereas in *Sclerospora graminicola* frequently a point somewhat above the middle shows the greatest breadth, and above this the spore is bluntly rounded, while below it

tapers gradually to a more narrowly rounded base (Pl. I, A, 5, 6, 7). In size there is relatively little difference, as was expressed in Kulkarni's measurements of 18 to 32 μ by 16 to 23 μ for the conidia on sorghum as opposed to 19 to 31 μ by 16 to 21 μ for those of Scl. graminicola and as is shown in the accompanying table 1, which by more adequate quantitative expression of sizes brings out the tendency to greater breadth in the conidia on sorghum. These characteristics of the conidial phase persist unaltered when the fungus develops on maize, so that, even on this host, which is in a tribe, Maydeae, separate from the Andropogoneae, to which sorghum belongs, the distinctive features are retained.

TABLE 1.—Comparison of sizes of the conidia of Sclerospora graminicola on Pennisetum typhoideum and of Scl. sorghi on Andropogon sorghum at Poona, India

	Length		Diameter				
Classes	Number o		Classes	Number of conidia in 400			
in μ	Andropogon sorghum	Pennisetum typhoideum	in μ	Andropogon sorghum	Pennisetum typhoideum		
13 to 14.9	0	0	13 to 14.9	0	22		
15 to 16.9	6	15	15 to 16.9	20	243		
17 to 18.9	9	63	17 to 18.9	29	82		
19 to 20.9	98	182	19 to 20.9	180	48		
21 to 22.9	112	77	21 to 22.9	113	5		
23 to 24.9	132	47	23 to 24.9	53	0		
25 to 26.9	40	14	25 to 26.9	5	0		
27 to 28.9	3	2					

The foregoing distinctions between Sclerospora graminicola and the species on sorghum are for the most part relative rather than absolute, quantitative rather than qualitative, variable rather than constant. Their appreciation always necessitates laborious comparisons and measurements of a large number of individuals; their expression in some instances requires compilation in tabular form. If between these 2 fungi these were the only differences, taken together they would serve as distinguishing features, and one would perforce use them, as one must, in separating various species in this genus and in related genera. Fortunately, however, this is not necessary, and these differences can be relegated to the position of merely supplementing and augmenting one absolutely clear-cut, qualitative distinction.

This distinction is that in the conidia of the fungus on sorghum the wall continues unaltered and quite unmodified across the apex (Pl. I, B, 5a-7a), while, in the case of *Sclerospora graminicola*, the end of the spore at maturity is thickened into a boss-shape protrusion of modified wall substance, the apical papilla of dehiscence (Pl. I, A, 1, 5, 6, 7). This structural

feature differentiates the fungus on sorghum with absolute distinctness from Scl. graminicola on Pennisetum and related hosts and would indeed distinguish it if the 2 species were to be found growing together on the same leaf of sorghum. Concomitant with this distinct structural difference, there is also an absolute difference in the method of germination of the spore, for in Scl. graminicola the content divides into zoospores that emerge through the softened apical papilla and swarm about, while the conidia of the fungus on sorghum invariably germinate by giving rise to 1 or 2 hyphae (Pl. I, B, 7a). That this is an absolute distinction is evidenced by the fact that only rarely in hundreds of cases observed and under conditions very unfavorable for normal development have the sporangia of Scl. graminicola ever been seen to send forth hyphae, and then only abortive, scanty growths (cf. Hiura 6, Weston 16), while in the case of the species on sorghum the conidia, without exception, germinate by hyphae, if at all.

The resting-spore phase of the fungus on sorghum usually matures, as in Sclerospora graminicola, after the conidial phase has declined. oogonia are formed as brown dots in the mesophyll between the fibrovascular bundles (Fig. 1, A). As the development of these spores proceeds, the tissue between the bundles is destroyed, resulting in a longitudinal splitting of the leaves along the veins into masses of tangled fibers (Fig. 1, B). This extensive destruction of the mesophyll, with the consequent shredding of the leaves, is one of the most distinctive symptoms of the downy mildew of sorghum and is also characteristic of Scl. graminicola on Setaria but not on Pennisetum typhoideum, in which case only the leaves enclosing the ears split into shreds at the tip. The spores themselves, in size, in the color and surface configuration of the thick enveloping oogonial wall, and in the diameter and wall thickness of the single oospore within, are similar to those of Scl. graminicola, as was originally emphasized by Butler and Kulkarni. This is further corroborated by the data on the measurements of oospores from different collections on different hosts summarized in table 2, although one of us (Weston) has found slight biometric differences in the different collections, but no absolute, clear-cut, qualitative distinctions have been brought out as yet.

The situation presented by this fungus on sorghum, then, is one of interest since in its cosporic stage it closely resembles Sclerospora graminicola, while in its conidial phase it is clearly distinct. The perfect or sexual stage, i.e., the cosporic phase, is, to be sure, the one emphasized in certain other groups as the basis of specific distinctions, yet it should be noted that, in the closely related genera Peronospora and Plasmopara, even in such early monographs as those of Fisher (4) and of Berlese (1) specific distinctions in many cases depended on conidial phases, while more recent work of Gäumann (5), Wartenweiler (13), and others has used in the separation of

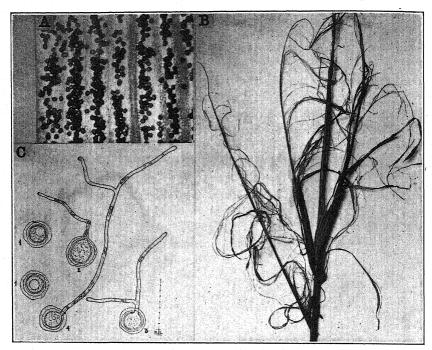


FIG. 1. A. Photomicrograph of a portion of leaf of sorghum, showing the linear arrangement of oospores in the mesophyll between the fibrovascular bundles. ×28. B. Specimen of sorghum infected by Sclerospora sorghi, showing the characteristic longitudinal splitting of leaves along the vascular bundles into masses of tangled fibers. ×½. C. 1. Resting spores, showing the single enclosed oospore. The content of the oospore is granular with 1 or 2 oil globules. 2. A germinating oospore with an unbranched germ tube. The wall of the germ tube is continuous with that of the oospore. 3 and 4. Same as 2, but the germ tube is branched. Camera-lucida drawings. Scale in microns. ×150.

species various morphological differences in the conidiophores and conidia, distinctions most of which are not absolute but decidedly relative and require expression quantitatively by large numbers of measurements of adequate populations.

When all the foregoing points are considered, the conidial phase of this Sclerospora of sorghum seems in its absolute lack of a dehiscence papilla in the apical wall of the conidia and their consequent invariable germination by hyphae, and in the definite basal cell, extensive branch system and longer sterigmata of the conidiophore, to furnish an adequate basis for its establishment as Scl. sorghi, a species distinct from Scl. graminicola.

PHYSIOLOGICAL DISTINCTIONS

In addition to the morphological distinctions noted above, this fungus on sorghum shows certain physiological differences as well, chiefly in its

TABLE 2.—Summarized measurements of oospores of Sclerospora sorghi and Scl. graminicola

Classes Scl. sorghi; Scl. sorghi; Scl. sorghi; Discratical statical static			Diameter				Width of wall	
number in $1,000$ On Setaria 1 On Pennisetum $viridis$ On Setaria 1 In μ μ 0 0 0 0 0.3 to 1.09 3 3 5 3 1.1 to 1.89 9 3 10 8 1.1 to 1.89 77 40 52 36 2.7 to 3.49 114 43 56 52 3.5 to 4.29 265 94 103 77 3.5 to 4.29 89 41 31 35 3.5 to 4.29 89 41 31 35 3 9 7 2 9 9 9 7 2 9 9 9 1 2 3 3 9 1 2 3 3	5	Scl. sorghi;	Scl. gram	inicola; number in	400	Hasses	Number	in 150
0 0 0 0 0.3 to 1.09 3 3 5 5 3 1.1 to 1.89 9 3 10 8 1.1 to 1.89 114 43 52 36 2.7 to 3.49 265 94 103 77 3.5 to 4.29 89 41 31 35 85 89 41 31 35 9 9 7 2 9 9 0 0 2 1 1	in µ	number in 1,000		On Pennisetum typhoideum	On Setaria viridis	in µ	Sel. $sorghi$	Scl. graminicola
3 3 5 3 1.1 to 1.89 9 3 10 8 1.9 to 2.69 77 40 52 36 2.7 to 3.49 114 43 56 52 3.5 to 4.29 265 94 103 77 3.5 to 4.29 89 41 31 35 89 41 31 35 9 7 2 9 0 1 2 3 0 0 2 1	23 to 24.9	0	0	0	0	0.3 to 1.09	14	15
$\begin{array}{cccccccccccccccccccccccccccccccccccc$	25 to 26.9	60	က	16	က	1.1 to 1.89	54	38
30.9 77 40 52 36 2.7 to 3.49 32.9 114 43 56 52 3.5 to 4.29 34.9 265 94 103 77 3.5 to 4.29 36.9 399 41 31 35 40.9 35 41 31 35 42.9 9 7 22 9 44.9 0 1 2 9 46.9 0 0 2 1	27 to 28.9	6	က	10	80	1.9 to 2.69	64	7.5
to 32.9 114 43 56 52 3.5 to 4.29 to 34.9 265 94 103 77 8.5 to 4.29 to 36.9 399 137 115 143 35 to 48.9 89 41 31 35 to 40.9 35 37 22 33 to 42.9 9 7 2 9 to 44.9 0 1 2 9 to 46.9 0 2 1	29 to 30.9	7.2	40	52	36	2.7 to 3.49	16	55
to 34.9 265 94 103 to 36.9 399 137 115 to 40.9 89 41 31 to 40.9 35 37 22 to 42.9 9 7 7 2 to 44.9 0 1 2 to 46.9 0 0 0 2	to	114	43	26	52	to	0 1	က
36.9 399 137 115 38.9 41 31 40.9 35 37 22 42.9 9 7 2 44.9 0 1 2 46.9 0 0 2	40	265	94	103	7.7			
38.9 89 41 31 40.9 35 37 22 42.9 9 7 2 44.9 0 1 2 46.9 0 0 2	35 to 36.9	399	137	115	143			
40.9 35 37 22 42.9 9 7 2 44.9 0 1 2 46.9 0 0 2	37 to 38,9	68	41	31	35			
42.9 9 7 44.9 0 1 46.9 0 0	39 to 40.9	35	37	 	33			
44.9 0 1 46.9 0 0	41 to 42.9	6	7	C 1	6			
45 to 46.9 0 0 2 1	43 to 44.9	0	-1	C 3	က			
	45 to 46.9	0	0	C 1	-			

range of hosts and restriction of parasitism and also in its difference of effect on the host.

One of the main difficulties in determining the host range of the sorghum Sclerospora has been the failure to obtain artificial infection of the different hosts with the conidia produced on the living plants of sorghum or with the oospores placed on the germinating seed in the soil. After a number of unsuccessful attempts, however, sorghum plants were successfully inoculated in 1929 with the oospore material of the fungus, which was in a finely powdered condition. Experiments were then made, in which the seedlings of maize, teosinte, Setaria italica, Pennisetum typhoideum, and sorghum were exposed to infection with the oospores of the sorghum Sclerospora, and parallel inoculations were made with Scl. graminicola. Also, because its oospores resemble those of Scl. sorghi, similar inoculations were made with Scl. noblei, a species recently described by Weston (15), which occurs on the wild Sorghum plumosum Beauvois in the uplands of New South Wales, Australia, whence material was kindly furnished by its discoverer, Dr. R. J. Noble. The seed of each host was sown separately in sterilized soil in 6-in. pots and water was supplied by capillarity from below. Fine oospore material was placed on each seed in the soil. In all cases control pots were provided, in which no inoculum was dropped on the seeds.

TABLE 3.—Summary of results obtained by inoculating different hosts with the oospores of Sclerospora sorghi, Scl. graminicola, and Scl. noblei

	Percentage of infected plants						
		Oospor	es of				
Host inoculated		Scl. gran	inicola				
	$Scl.\ sorghi$	on Pennisetum typhoideum	on Setaria italica	Scl. noblei			
Andropogon sorghum	32.2	0	0	0			
Pennisetum typhoideum	0	21.4	0	0			
Setaria italica	0	0	46	0			
Euchlaena mexicana	16.0	0	14	0			
Zea mays (Golden Bantam)	45.0	0	0	0			
Zea mays (Philippine variety)	6.8	0	0	0			
Zea mays (Indian variety)	44.0	0	0	0			

The results are summarized in table 3 and show that the seedlings of sorghum, teosinte, and 3 varieties of maize were successfully inoculated

with the oospores of the sorghum Sclerospora. A more detailed account of the inoculation experiments with maize and teosinte has been given elsewhere (12). Negative results, however, were obtained with Scl. noblei and Scl. graminicola, the latter producing infection only on its hosts and on teosinte when the physiologic form on Setaria italica was used to inoculate In teosinte and maize the characteristic chlorosis of the leaves was noticed about a fortnight after inoculation, the symptoms in sorghum appearing as early as the 9th day after the seed was exposed to infection. When placed under a bell jar, the affected plants produced abundant conidial fructification within a week after the appearance of the first symptoms of chlorosis. These plants showed pale yellow blotching on the 2nd and 3rd leaf, and, as the seedlings developed, the other leaves also became infected. The conidia of Scl. sorghi thus produced germinated profusely by hyphae at 14-16° C., the minimum and maximum temperatures of germination being 10 and 30° C., respectively. The results on germination are tabulated in table 4.

TABLE 4.—Relation of temperature to germination of conidia of Sclerospora sorghi

Temperature (° C.)	Percentage of germination in tap water
6–8	0
8–10	0
10–12	50.0
12–14	88.0
14-16	90.0
16–18	85.0
18–20	65.0
20–22	50.0
22–24	52.0
24-26	50.0
30–31	0

The method used in germinating the oospores was essentially similar to that recently described by Hiura (7). The finely powdered spore material was dusted on blotting-paper supported partly on a wad of wet cotton wool placed at the bottom of a Petri dish. The lid of the Petri dish was lined inside with wet cotton wool, so that, on closing the lid, an atmosphere of high humidity was obtained within the Petri dish.

The dish was held at room temperature (23-25° C.) for about 48 hours. At the end of this period the spores had germinated by hyaline, unseptate germ tubes, which were usually branched (Fig. 1, C, 3 and 4); in some cases no branching was observed (Fig. 1, C, 2). The content of the oospore is hyaline, with a finely granular matrix, in which occur dense granular

bodies and 1 or 2 large masses of oil globules, which may be central or eccentric in position (Fig. 1, C, 1); but the germinating oospores show the granular bodies without the oily substance. In germination the wall of the oospore pushes out through the oosporial wall and grows into a germ tube, into which the contents of the oospore finally flow out. The germ tube varies in width from 2.5 to 8.3 μ , the average width being 4.4 μ .

DIAGNOSIS

The foregoing considerations seem to justify establishing this fungus as a distinct species with emended diagnosis as follows:—

Sclerospora sorghi (Kulk.) Weston and Uppal.

Syn. Sclerospora graminicola var. Andropogonis sorghi Kulkarni 1913 in Mem. Dept. Agr. India Bot. Ser., V. 5, No. 5, p. 268–273, pl. 6–7, 1 colored.

Conidiophores erect, spreading, comprising basal cell, main axis and more or less complex, usually dichotomously branched, expanded top. The basal cell knobbed or bulbous at the bottom, then of fairly uniform diameter (7–9 μ), for a length of approximately 100 to 150 μ until delimited usually by a complete septum, more rarely by a partial, ring-like thickening. The main axis expanding above to a diameter of 15 to 25 μ , and usually less than or equal to the basal cell in length, i.e., extending about 80 to 150 μ , from the septum of the basal cell to the beginning of the branch system. The branch system comprising rapid successions of short, stout dichotomies usually involving primary, secondary, and tertiary branches terminating in tapering sterigmata usually about 13 μ long, the branches so arranged that the conidia borne on their tips lie approximately in a hemispherical plane.

Conidia suborbicular, varying from 15 to $28.9~\mu$ x 15 to $26.9~\mu$, most frequently 21 to $24.9~\mu$ x 19 to $22.9~\mu$, under natural conditions. Conidia hyaline, with a thin wall, continuous at the apex, unmodified, and quite without any papilla of dehiscence, hence germinating invariably by hyphae.

Oogonial stage resembling that of Sclerospora graminicola in general structural characteristics, such as the thick, irregularly polygonally-angled oogonial wall closely enveloping the single, hyaline, spherical oospore within, but differing slightly from Scl. graminicola on Pennisetum in effect on the host in that oospores, which develop chiefly within elongate reddish discolored areas in the mesophyll between the fibrovascular bundles, cause marked disintegration of the leaf tissue into tangled fibers. Oospores spherical, the majority being 31 to 36.9 μ in diameter, the mode being 35 to 36.9 μ , extremes ranging from 25 to 42.9 μ ; wall a light shade of Mars Yellow (of Ridgway's "Color Standards"), most frequently from 1.1 to 2.7 μ thick, extremes ranging from 0.3 to 4.3 μ ; content finely granular with

masses of oil globules, central or eccentric in position; germination by means of unseptate, usually branched, hyaline germ tube, averaging 4.4 μ in width, extremes ranging from 2.5 to 8.3 μ .

Occurring in both conidial and oogonial phase principally on sorghum (Andropogon sorghum) in the Bombay and Madras Presidencies, India, and rarely in its conidial phase on maize (Zea mays) near Poona, India, and also in Giza, Lower Egypt. Inoculated successfully on teosinte (Euchlaena mexicana). Very destructive to sorghum in Bombay and Madras Presidencies.

SUMMARY

The history of this fungus, which was first recognized by Kulkarni, in 1913, as a variety, *Andropogonis sorghi*, of *Sclerospora graminicola* is reviewed.

Significant structural details of the conidial phase of this fungus on sorghum are compared with those of typical Scl. graminicola on Pennisetum and Setaria. The fungus on sorghum differs distinctively in its absolute lack of a dehiscence papilla in the apical wall of the conidia and their consequent germination by hyphae, and in the definite basal cell, extensive branch system, and consequent arrangement of the conidia in a hemispherical plane on the longer sterigmata of the conidiophores. On these features the fungus is separated from Scl. graminicola to specific rank as Scl. sorghi.

The oogonial phase resembles that of *Scl. graminicola* in general characteristics, but cross-inoculation experiments, using oospore material as inoculum, show that *Scl. sorghi* will not inoculate Setaria and Pennisetum, nor will *Scl. graminicola* from Pennisetum or Setaria inoculate sorghum, in spite of successful inoculations from each of these hosts to such remotely related Gramineae as *Euchlaena mexicana*. The results of these inoculations, which offer corroborative evidence for recognizing the fungus as a distinct species, are described and tabulated.

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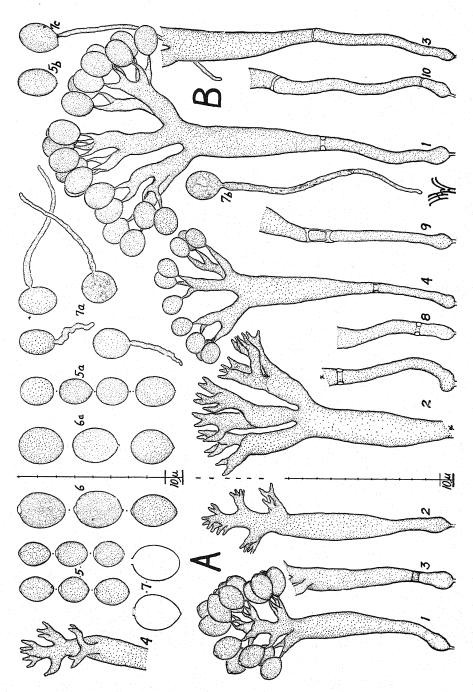
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EXPLANATION OF PLATE I

Comparison of Sclerospora graminicola (Sacc.) Schroet. A, 1-7, with Sclerospora sorghi, n. sp. B, 1-10. Drawings made with camera lucida from preserved material collected at Poona, India, during period of abundant conidiophore production. Magnifications apply to the present figures. Scales in microns.

A. Sclerospora graminicola. A, 1. Mature sporangiophore showing short, stocky habit, lack of basal cell, and main axis continuing into branch system with short laterals bearing ovate sporangia in groups. ×375. (cf. B, 1). A, 2. Similar sporangiophores after loss of sporangia showing typical habit, branch system, and sterigmata. ×375. (cf. B, 2). A, 3. Main axis of similar sporangiophore showing unusual occurrence of a true basal cell. ×375. (cf. B, 3). A, 4. Upper portion of a sporangiophore with branch system less typical yet showing continuance of main axis. ×375. A, 5. Mature sporangia of common shapes and sizes with characteristic papilla modification of apical wall through which zoospores emerge at germination. ×375. (cf. B, 5). A, 6. Similar sporangia enlarged, showing basal apiculus of attachment to sterigma, not to be confused with modified apical papilla. ×500. A, 7. Empty sporangial cases from which zoospores have emerged. ×500. (cf. germination by germ tubes in B, 6).

B. Sclerospora sorghi. B, 1. Mature conidiophore showing slender, isodiametric, relatively long basal cell, well developed "deliquescent" branch system, bearing true conidia in hemispherical arrangement. ×375. (cf. A, 1). B, 2. Similar conidiophore (after discharge of conidia) with characteristic spreading branch system and definite



basal cell. ×375. (cf. A, 2). B, 3. Main axis of conidiophore showing characteristic basal cell about equal in length to main axis above. ×375. (cf. A, 3). B, 4. Young depauperate conidiophore approximating in size those of Scl. graminicola, yet retaining characteristics of Scl. sorghi, i.e., basal cell, expanding branch system, and hemispherically placed conidia. ×375. (cf. A, 1). B, 5. Representative mature conidia. Note lack of any apical papilla. ×375. (cf. A, 5). B, 6. Similar conidia enlarged. ×500. (cf. A, 6). B, 7. Conidia showing characteristic germination by hyphae. ×375. (cf. A, 7). B, 8. Nontypical short, broad, basal cell resembling those rarely occurring in Scl. graminicola. ×375. (cf. A, 3). B, 9 and 10. Basal cells showing aberrant septations occasionally seen. ×375.

TWO NEW HOSTS OF THE DOWNY MILDEW OF SORGHUM IN BOMBAY¹

B. N. UPPAL AND M. K. DESAI

Sclerospora graminicola var. Andropogonis-sorghi Kulk. was described by Kulkarni in 1913 as the cause of the downy mildew of sorghum in the Bombay Presidency. He considered this fungus as a variety of S. graminicola (Sacc.) Schroet, showing distinct differences in its conidial stage and in its ability to infect Pennisetum typhoideum Rich. Until recently (4, 7), therefore, sorghum was the only host reported for this mildew. However, in 1929 some varieties of maize growing in the students' area on the College Farm, Poona, were badly affected by a downy mildew, and, since this disease had never been previously reported in the Bombay Presidency, its presence on maize was of special interest and led to the examination of the affected plants. It was surprising to find a very close resemblance between the conidia of the downy mildew on maize and those of the sorghum Sclerospora. It was, therefore, decided to make a detailed study of the downy mildew on maize and to determine whether it was possible to obtain artificial infection of this host with the oospores of S. graminicola var. Andropogonissorahi.

COMPARATIVE STUDY OF THE SCLEROSPORA ON SORGHUM AND MAIZE

Field symptoms. As observed in the field, the symptoms and the general effects of the downy mildew on the individual plants of sorghum and maize were the same. The leaves of the affected plants were pale yellow and were covered with the conidial stage of the fungus. These symptoms were noticeable when the plants were about 5 weeks old. At a later stage sorghum plants showed the characteristic shredding of the affected leaves, but no such effects were produced in maize, which continued to support the conidial fructification on the pallid areas of the leaves. The fungus did not produce oospores in the leaf tissues of the maize.

Infection experiments. Since it has been possible to infect sorghum with the oospores of Sclerospora graminicola var. Andropogonis-sorghi (6), experiments were made to determine whether the seedlings of maize and teosinte (Euchlaena mexicana Schrad.) could be successfully inoculated with this variety. Teosinte was included in these studies, since Butler has reported this as a host of S. graminicola on Pennisetum typhoideum, which has, however, recently been shown by the writers (8) not to infect teosinte under controlled conditions. Butler (2, pp. 193 and 223) has already expressed a doubt as to the identity of the downy mildew on teosinte

¹ Published with the approval of the Director of Agriculture, Bombay Presidency.

TABLE 1.—Results obtained from exposing 2 varieties of maize to infection by oospores of Sclerospora graminicola var. Andropogonis-sorghi, 1929

Plants exposed to infection	Oospores from	Number of plants exposed	Percentage of plants infected
Zea mays (local)	Andropogon sorghum	6	50.0
Control ^a	Nil	6	0.0
Z. mays (Golden Bantam)	A. sorghum	7	57.0
Control ²	Nil	7	0.0

a Control plants remained healthy in all cases.

but has suggested that S. mydis (Rac.) Butl. might be the species concerned because of the absence of the oosporic stage on this host. However, since this species has not been recorded in Bombay, the identity of the downy mildew on teosinte first seen by Butler (1), in 1905, on the Poona Government Farm, became very doubtful.

1929 experiment. On August 15, 1929, a variety of maize—sweet corn (Golden Bantam)—and local maize were sown in sterilized soil in 6-inch pots. The seed bed was watered before the seed was sown. In each pot small holes, about 2 to 3 in. deep, were made in the seed bed, and oospore material of the sorghum Sclerospora was dropped at the bottom of each hole. A seed was sown in each hole, and the oospore material was again dropped on each seed. The soil was restored on the seed. To prevent the oospores from being washed down, the pots were allowed to stand in China dishes full of water so that water was supplied to the germinating seeds by capillarity, thus ensuring against the least disturbance to the physical contact of the oospores with the seed. In each case control pots were provided in which no inoculum was dropped.

A reference to table 1 will show that the seedlings of sweet corn (Golden Bantam) and local maize were successfully inoculated with the oospores of the downy mildew of sorghum. In Golden Bantam the characteristic chlorosis of leaves was noticed 14 days after the seed was exposed to infection; but, in the seedlings of local maize, these symptoms did not appear until the 19th day after inoculation. When placed under a bell jar, the affected plants produced conidial fructification within a week of the appearance of the first symptoms of the downy mildew. These plants showed pale yellow blotching on the 2nd or 3rd leaf, and, as the seedlings developed, the other leaves also became infected. Sporulation was obtained also on

leaves placed in Petri dishes lined with wet blotting paper and exposed to tap water at 17–18.5° C. for about 9 hours. There was, however, profuse sporulation when these leaves were left overnight in Petri dishes at room temperature (20–22° C.). In all tests conidia germinated by the protrusion of 1 or 2 germ tubes.

1930 experiment. Besides the varieties of maize tested in 1929, teosinte and Philippine maize were included in the trials made in 1930. The method of inoculation was essentially the same as described above. In addition to the 6-inch pots, wooden flats were filled with sterilized soil and the seed beds were thoroughly wet down before the seeds were sown. In each seed bed furrows were opened, and the seed was moistened, rolled in fine oospore material and sown in the furrows. Oospores were again dusted on the seeds through a fine muslin cloth. The soil was finally restored to the furrows. In all cases water was supplied by capillarity. Control plants were provided for each series. The seeds germinated well in all cases.

TABLE 2.—Results obtained from exposing varieties of maize and teosinte to infection by oospores of Sclerospora graminicala var. Andropogonis-sorghi, 1930

Plants ^a exposed to infection	Oospores from	Number of plants exposed	Percentage of plants infected
Zea mays (local)	Andropogon sorghum	54	38.8
" (Philippine)	"	29	6.8
" (Golden Bantam)		29	34.4
Euchlaena mexicana		50	16.0

a Control plants remained healthy in all cases.

It will be seen from table 2 that all the varieties of maize and teosinte were infected with the oospores of the downy mildew of sorghum, although the percentage of infection in the Philippine maize was low. In a few cases the symptoms were first noticed as early as the 14th day after inoculation, but in some others they did not appear before the 25th day or even before the lapse of a month. Sporulation was readily obtained on affected plants placed under a bell jar or on leaves in Petri dishes subjected to tap water. The condia germinated by the formation of 1 or 2 germ tubes.

MORPHOLOGICAL CHARACTERS

After it had been ascertained that the downy mildew of sorghum can infect maize under controlled conditions, it was decided to make a comparative morphological study of the conidial phase of the mildew as it

occurs naturally on maize and sorghum in Bombay. The material for study was obtained from plants growing in the field during the time of optimum conidiophore production at night between 3 and 4 a. m. The leaves of naturally infected plants were wiped clean of their previous sporulation with wet cotton wool, in the evening, and a rich crop of conidia was obtained in the following night a few hours after midnight. The conidia were mounted in water and the measurements were invariably made before sunrise.

Conidiophores. In general appearance the conidiophores of the Sclerospora on sorghum were exactly similar to those of the downy mildew on maize. (Fig. 1, 1, 2 and 1a, 2a). Each was provided with a basal cell, which had usually 1 septum separating it from the rest of the main axis. (Fig. 1, 1 and 1a, 2a). In the Sclerospora on sorghum sometimes 2 septa were seen in the basal cell. (Fig. 1, 2 and 3). The average length of the

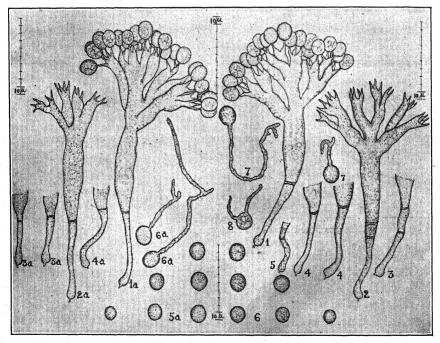


Fig. 1. Comparison of Sclerospora graminicola var. Andropogonis-sorghi on Andropogon sorghum and Zea mays: On A. sorghum—1, a well-developed conidiophore with a basal cell; 2, 3, basal cells with 2 septa; 4, basal cells of conidiophores of length normally encountered; 5, a part of a conidiophore showing a basal cell much shorter than normally encountered; 6, conidia showing range of shapes and sizes; 7, 8, germinating conidia with 1 or 2 germ tubes. On Z. mays—1a, same as in 1; 2a, a sparsely branched conidiophore with conidia fallen off; 3a, 4a, same as in 4; 5a, same as in 6; 6a, germinating conidia with 1 germ tube.

conidiophores of Sclerospora graminicola var. Andropogonis-sorghi was about $200 \,\mu$.

Conidia. In shape and size the conidia of the Sclerospora on maize agreed very closely with those of S. graminicola var. Andropogonis-sorghi. (Fig. 1, 6 and 5a). They are suborbicular and lack an apical papilla of dehiscence. They, therefore, invariably germinate by the formation of 1 or 2 germ tubes. (Fig. 1, 7, 8, and 6a).

In order to obtain a correct impression of the conidial dimensions, 400 conidia, each, from maize and sorghum were measured and arranged in a series of classes. It will be seen from table 3 that the measurements of the

TABLE 3.—Summarized	measurements	of conidia	of the	e Sclerosporas on	sorghum
	and	maize			

	Le	$_{ m ngth}$		Wid	lth
Classes in µ		of conidia 400	Classes in µ	Number o	
	Sorghum	Zea mays		Sorghum	Zea mays
15 to 16.9	6	7	15 to 16.9	20	16
17 to 18.9	9	8	17 to 18.9	29	27
19 to 20.9	98	108	19 to 20.9	180	180
21 to 22.9	112	115	21 to 22.9	113	114
23 to 24.9	132	114	23 to 24.9	53	50
25 to 26.9	40	45	25 to 26.9	5	13
27 to 28.9	3	3	27 to 28.9	0	0

length and width of conidia produced on sorghum are in complete agreement with similar measurements of conidia taken from maize.

The biometrical constants of the conidial dimensions are given in table 4. An examination of the data will show that the standard deviations for length and width in the case of the Sclerosporas on sorghum and maize are almost the same. For example, the standard deviation for the length of conidia is 2.21 ± 0.052 in the case of the Sclerospora on sorghum and 2.26 ± 0.053 in the case of the fungus on maize, and for width the deviations are 2.02 ± 0.048 and 2.07 ± 0.049 , respectively. Likewise, the coefficients of variability, both for length and width, also agree very closely. Also, the data presented in table 5 indicate that the differences in the means of length and width of the conidia of both the Sclerosporas are very insig-

TABLE 4.—Biometric constants for length and width of the Sclerosporas on sorghum and maize

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	Lenş	gth		
Sclerospora on	Mean	Mode	Standard deviation	Coefficient of variability
Andropogon sorghum	22.43 ± 0.072	23.9	2.21 ± 0.052	9.8 ± 0.23
Zea mays	22.34 ± 0.07	21.9	2.26 ± 0.053	10.1 ± 0.24
	Wic	lth		
Andropogon sorghum	20.82 ± 0.068	19.9	2.02 ± 0.048	9.7 ± 0.23
Zea mays	20.97 ± 0.069	19.9	2.07 ± 0.049	9.8 ± 0.23
		·		

nificant; that is, the odds against the occurrence of the differences as great as or greater than 0.941 in the case of length and 1.535 in the case of width are about 1:1 and 2.21:1, respectively. In other words, the differences for length and width of the conidia of the Sclerosporas on sorghum and maize have no statistical significance and may be due to random fluctuation, thus indicating that the 2 fungi are identical.

DISCUSSION

The experimental data above presented clearly indicate that the downy mildew on maize, which is found occurring naturally in the Bombay Presidency in certain seasons, is identical with *Sclerospora graminicola* var. *Andropogonis-sorghi* both in its effects on the host and in the morphological characters. This fungus must now be added to the list of 7 species of Sclerospora already known to attack maize.

The occurrence of the sorghum Sclerospora on maize is of special interest, since the development of the sexual stage, which is a prominent char-

TABLE 5.—Differences in means of the Sclerospora on sorghum and maize relative to the probable errors

Differen	ce in means	divided b	e in means y probable difference	Odds (approximately)	
Length	Width	Length	Width	Length	Width
0.095 ± 0.10	0.15 ± 0.097	0.941	1.535	1.00:1	2.21:1

acter of this fungus on sorghum, is completely suppressed in maize. This phenomenon, however, is not peculiar to the sorghum Sclerospora, since Sclerospora graminicola, occurring on Setaria viridis (L.) Beauv. and others, has also been shown not to produce oospores in maize (5). The question arises as to how a species of Sclerospora that normally produces the oogonial stage can be so altered by its passage from its host to maize that its sexual stage is suppressed in the latter host. Clinton (3) has reported a similar relationship in Phytophthora infestans (Mont.) de Bary, which can be induced to produce oospores more readily on oatmeal agar than on other media. However, the relation of food materials to the development of the oogonial stage in the species of Sclerospora cannot be determined until it is possible to grow these fungi in artificial culture.

In 1905 Butler first saw a downy mildew on teosinte, Euchlaena mexicana, on the Government Farm, Poona. Since Sclerospora graminicola was commonly found on *Pennisetum typhoideum* growing in the vicinity, Butler was led to believe this mildew occurred on teosinte in Bombay, a statement he later modified, as this mildew was not seen to produce the oogonial stage in the latter host. It is very likely that Butler (1) came to this conclusion because he could not find the conidial stage of the Sclerospora on sorghum, which he then considered to agree with the species on Pennisetum. It, therefore, seems very reasonable to believe that the mildew on teosinte, seen by Butler in 1905, was, in fact, the form on sorghum, for it has recently been shown by the writers (8) that S. graminicola is split into specialized races and that the form on P. typhoideum fails to infect teosinte. Moreover, this conclusion is justified in view of the fact that S. graminicola var. Andropogonis-sorghi is found very commonly on sorghum in Bombay, while S. maydis (Rac.) Butl., later considered by Butler (2, pp. 193 and 223) as the cause of the downy mildew of teosinte, has never been recorded in this Presidency.

SUMMARY

- 1. The downy mildew of maize occurring naturally on this host in Bombay has been found to be caused by *Sclerospora graminicola* var. *Andropogonis-sorghi*. In the conidial stage it is not possible to distinguish between the mildews on sorghum and maize. The sexual stage is prominent on sorghum, resulting in the characteristic shredding of leaves, but it is completely suppressed in maize.
- 2. Maize and teosinte (Euchlaena mexicana) have been infected with the oospores of S. graminicola var. Andropogonis-sorghi under controlled conditions.
- 3. A comparative morphological study of the conidial phase of the mildew as it occurs naturally on maize and sorghum in Bombay has been made.

In general appearance the conidiophores are alike, and each is provided with a basal cell. The biometrical measurements of the conidia of the mildews on maize and sorghum have shown that the differences in length and width of conidia are not statistically significant, thus indicating that the 2 fungi are identical.

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CHEMICAL TREATMENT OF PECAN ROSETTE¹

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INTRODUCTION

Pecan rosette, a disease apparently of nonparasitic origin, is present in every state and on almost every soil type where pecans are grown commercially. In some of the Southeastern States, hundreds of acres of pecan orchards made unprofitable by this disease have been abandoned and relatively few of the commercial plantings in the whole pecan territory are entirely free from it. In the Southwestern States where the commercial plantings are more recent, and more especially on the basic soils, as high as 95 per cent of the trees are rosetting in some localities. This is true of nursery trees as well as of planted orchards. In spite of the fact that growers are using more care in selecting orchard sites as well as taking better care of their orchards, rosette is now a very serious problem to nurserymen and orchardists alike.

Yellow mottling, or chlorosis, and crinkling of the leaves are characteristic of rosette. In orchards the tops of trees seem to be first affected, with a gradual spread to the lower branches. The disease progresses from a mild chlorosis of the leaves to a dwarfing of the leaves, shortening of the internodes, and, finally, to death of twigs and branches. While few, if any, trees die from rosette, severely rosetted trees are generally nonproductive and become so weakened that they die from attacks or borers or from other causes.

Rosette occurs under varying conditions on both residual and alluvial soils. Eroded areas, a tight subsoil condition, light soil textures, and deficiencies in organic matter seem to favor the occurrence of rosette on the residual soils. A high water table and soils of light texture favor rosette on the alluvial bottoms. Well-drained, fertile, medium to heavy soils types are generally free from rosette except under special conditions. On the alluvial soils of Louisiana, rosette occurs regardless of soil type when trees are growing near old building sites, near negro cabins, in extremely fertile garden soils, in barn lots, and near pigpens.

A varietal resistance or susceptibility to rosette seems to exist. The Stuart is the most susceptible, followed closely by the Frotscher and Van Deman varieties, while the Moneymaker seems to be highly resistant. The writers have never seen native pecan trees rosetting in their natural state in the woods. Native pecans left standing and brought under cultivation when

¹ Cooperative investigations between the Bureau of Chemistry and Soils and the Bureau of Plant Industry, U. S. Department of Agriculture, Washington, D. C.

the woods were cleared away have rosetted severely. Certain culture practices seem to favor the development of rosette, while others seem to hold it in abeyance or affect a cure.

REVIEW OF LITERATURE

Orton and Rand (4), in 1914, reported that no parasitic organism was associated with this disease. Their investigations indicated that the trouble lay in the soil, but whether the cause was physical, chemical, or biological was not indicated.

In 1919, McMurran (3), who worked on rosette from 1914 to 1920, reported that from "10 to 20 per cent of the pecans planted in orchards in the southeastern United States have shown this trouble in a more or less marked degree." After making a survey of pecan orchards from Florida to eastern Louisiana, McMurran also reported in the same publication that "while the disease was seen to be present on a wide range of soil types and under various conditions of cultivation and fertilization, one fact seemed to stand out prominently. A very large proportion of the diseased trees, probably 90 per cent or more, was found on the hilltops and slopes. Occasional cases or groups of cases were on bottom lands, but all of these that have been examined have been found to be growing in deep sand or else in a clay or sand clay underlain at 2 to 3 feet by sand. It was observed also that wherever the conditions were such as to produce any quantity of rosette, the weeds or crops growing in the tree rows had a stunted, yellow, unthrifty appearance as compared with those growing among healthy trees, . . . On the other hand, trees planted in low places in which humus and fertility had accumulated from year to year, were uniformly vigorous, thrifty, and free from disease."

McMurran further reported that "While apparently contradictory cases occasionally are seen, 90 per cent or more are surrounded by conditions that plainly indicate a deficiency of humus, plant-food material, and moisture. Either where the soil or topography is such that there is a deficiency of this combination, or where the tree's condition is such as to interfere with its obtaining an adequate supply of plant-food material, the result appears to be the same, *i.e.*, a yellowing of the foliage, a shortening of the internodes, a pushing out of dormant buds, and in many cases a dying back of the twigs and branches."

In 1922, Rand (5) concluded that rosette is "more in agreement with the infectious type of chloroses, including the yellows and mosaic groups, than with those chloroses known to be caused directly by soil or climatic conditions."

Skinner and Demaree (6), in their experimental work with rosette in southern Georgia, were able to bring badly rosetted trees that were growing

on soils low in organic matter back to normal by growing and turning under two green manure crops each year. Chemical fertilizers as used in the experiment had no influence in increasing or decreasing rosette.

EXPERIMENTS IN CONTROL

As shown by the literature as well as observation by the writers, the evidence very strongly favors the view that rosette is of non-parasitic origin

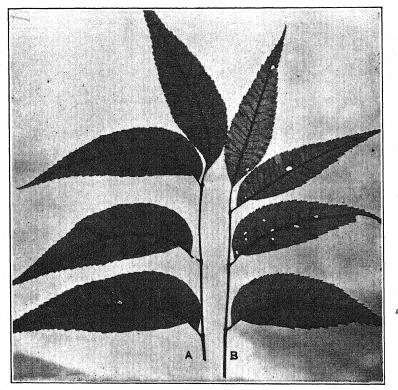


Fig. 1. A. Part of a pecan leaf that, 30 days before, had been dipped in a 1 per cent ferric sulphate solution. B. Part of a nontreated leaf from the same terminal as A. A is normal but B is almost devoid of chlorophyll.

and that its development depends on the presence of some soil factor or factors at present not definitely known. The disease does not seem to spread from badly rosetted trees to healthy ones, though their branches may be interlocking. Most of the trees in a locality seem to rosette simultaneously. Rosetted leaves and their extracted juices, when placed on healthy leaves, have failed to produce rosette.

Because of the increasing importance of rosette in the Southwestern States, the writers outlined a series of experiments in 1930 and expanded them in 1931, with the hope of determining the cause of rosette. In these experiments, three different methods were used: first, culture practices; second, applications of chemicals to the soils; and third, application of chemicals in solution to the leaves.

The culture practices have consisted in (1) clean cultivation; (2) the growing of a cover crop that was allowed to stand. No results have been obtained, since the experiment has run but one season.

Calcium sulphate, manganese sulphate, magnesium sulphate, and potassium chloride were applied to both hill and bottom-land soils in April, 1930

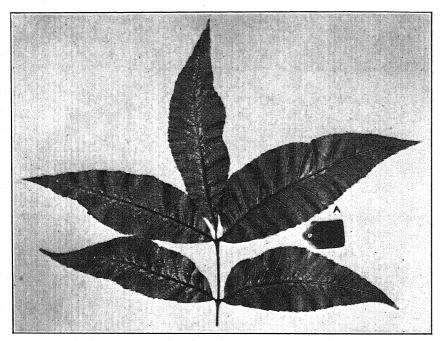


Fig. 2. Part of a pecan leaf. One leaflet, A, received an application of ferric sulphate solution (0.8%). At the time the photograph was taken, 12 days after treatment, the treated leaflet was almost normal and increased in size over its companion nontreated leaflet.

and 1931, and plowed under; in addition, ferrous and ferric sulphate were placed in trenches in August, 1931, on bottom-land soils. None of these treatments has shown any apparent beneficial effects up to the present time.

Calcium sulphate, manganese sulphate, magnesium sulphate, potassium bromide, potassium sulphate, aluminum sulphate, boric acid, hydrochloric acid, and sulphuric acid, in varying strengths, were sprayed on trees May 26, 1931, without any apparent beneficial results. Ferric sulphate and

ferric chloride, sprayed on Schley and Stuart trees, August 1, 1931, either improved the condition of rosetted leaves or brought them back to normal, depending on their stage of growth.

Rosetted leaves were treated with both ferric sulphate and ferric chloride in strengths varying from 0.1 to 1 per cent. Improvement was noted in the more dilute applications ranging from 0.1 to 0.5 per cent. Complete recovery was effected with strengths ranging from 0.6 to 1 per cent. Slight

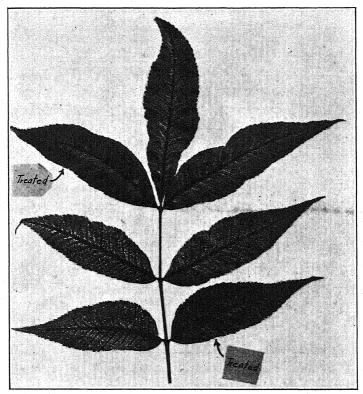


Fig. 3. A pecan leaf. Ferric sulphate solution (0.8%) was applied to the two leaflets indicated by arrows. The upper treated leaflet has almost completely recovered and the lower one shows improvement. Photographed 12 days after treatment.

burning with subsequent recovery resulted from the use of 0.8 to 1 per cent dilution. An effort was made to prevent the burning effect of the stronger solutions by neutralizing their acidity with lime. The results of this experiment were an increase in burning and no improvement of the rosette condition. Figures 1, 2, 3, and 4 show some of the results obtained by applying solutions of iron salts to pecan leaves.

DISCUSSION AND CONCLUSIONS

The evidence of a single season's treatments must, of course, be recognized as inconclusive, partly because spraying treatments were carried out in the latter part of the growing season rather than throughout the entire growing period and partly because of the possibility of the mid- or late-season treatments' masking chlorotic symptoms that might become apparent in the following season. Admitting the possibility of contradictory results in large-scale experiments and on other soil types and other varieties, it appears that the applications of solutions of iron salts to rosetted leaves of Stuart and Schley varieties cured or, at least, lessened the severity of rosette on trees growing in residual and in alluvial soils.



Fig. 4. Left, a rosetted pecan terminal that had been dipped in a 1 per cent ferric chloride solution with a normal leaf that developed after treatment; right, a near-by nontreated rosetted terminal. Photographed 45 days after treatment.

These findings would seem to indicate that pecan rosette is related to iron chlorosis. Iron chlorosis of other plants due to a high lime content of the soil has been known for years. As early as 1843, E. Gris (2) of France first found that iron sulphate applied to chlorotic plants through roots or leaves caused them to become green. Since that time, treatment of chlorosis with iron salts has been more or less common in Europe. In recent years (1) treatment of pears, peaches, apples, citrus, and other plants in the United States for lime-induced chlorosis has been much practiced. Pecan rosette, however, occurs on both acid and basic soils. Many facts have yet

to be brought to light and correlated before the cause of rosette can be satisfactorily explained. With work and experiments now in progress, the writers hope to throw more light on the cause of rosette and develop methods for curing or preventing this disease.

SUMMARY

Pecan rosette is apparently a nonparasitic disease of wide-spread distribution occurring on both residual and alluvial soils. A varietal resistance or susceptibility to rosette seems to exist. The Stuart is the most susceptible, while the Moneymaker seems to be highly resistant.

The writers were able to improve old rosetted leaves and bring the young ones back to normal by dipping or spraying them with a solution of ferric sulphate or ferric chloride, ranging in strength from 0.6 to 1 per cent. These findings would seem to indicate that pecan rosette is a condition of iron chlorosis.

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EFFICIENCY OF OILED WRAPS IN THE COMMERCIAL CONTROL OF APPLE SCALD

NEIL E. STEVENS AND NELLIE W. NANCE

That the commercial control of plant diseases frequently fails to approximate the degree of control obtained in experiments is a common observation. Relatively little information is available as to the actual results obtained in commercial practice, due largely to the difficulty of accurately measuring such results. The intensive surveys of 1929 and 1930 demonstrated that to an extent not previously recognized the commercial control of stinking smut of wheat often falls below experimental control.¹

The present paper is an attempt to evaluate the actual commercial efficiency of the oiled wraps in the control of apple scald, as developed by Brooks and his associates.² The information contained in the inspection certificates of the Food Products Inspection Service of the Bureau of Agricultural Economics, United States Department of Agriculture, forms the basis of this study. The summary is confined to the boxed apples of Washington State, since in this region the control measure was most promptly and generally adopted.

THE "OILED-WRAPS" METHOD OF CONTROL AND ITS ADOPTION

As is well known, practically all of the apples shipped in boxes from the Pacific Northwest of the United States have long been packed in individual paper wraps. The method for the control of apple scald here referred to is the use of paper impregnated with mineral oil instead of the untreated paper formerly used.

Several influences combined to give this control method prompt and wide application. The disease is easily distinguished, and had long been recognized as serious. The control method involved only a slight change in the commercial handling of boxed apples. The discoverers had the confidence of the industry and were actively engaged in field work in large producing centers. The industry itself is highly organized in the regions from which most of the boxed apples are shipped.

A statement of the rate of adoption is found in Brooks's paper of August, 1924. In the 1922 crop, about 600,000 boxes of apples in the North-

¹ Haskell, R. J., R. C. Rose, W. E. Brentzel, E. A. Walker, and Waldo Kidder. Why so much smut in spring wheat? U. S. Dept. Agr., Bur. Plant Indus., Plant Dis. Rptr. Sup. 77: 97-138. 1930. (Mimeographed).

² Brooks, Charles, J. S. Cooley and D. F. Fisher. Apple scald and its control. U. S. Dept. Agr. Farmers' Bul. 1380. 1923.

³ Brooks, Charles. Apple scald. Off. Proc. Inter. Apple Shippers' Assoc. 29: 154-158. 1924.

west went into storage in oiled wraps. In the summer of 1923, about 15,000,000 bushels were so treated. By 1924, the use of oiled wraps was almost universal in Washington, and had been adopted to some extent throughout the apple-growing regions of the country. For the barrelled fruit the method was modified by the use of shredded paper, scattered among the apples.

COMMERCIAL RESULTS IN THE CONTROL OF APPLE SCALD IN THE STATE OF WASHINGTON

As indicated in the preceding paragraph, a small proportion of the Washington apple crop was packed in oiled wraps in 1922, a much larger amount in 1923, and by 1924 the method had been generally adopted. Since that time it has continued in popularity and has become a part of regular commercial practice in that region.

The information on scald in apples during this interesting period, obtainable from the inspection certificates of the Food Products Inspection Service, is summarized in table 1. Calculated on the basis of all cars

TABLE 1.—Scald and	blue	$mold\ in$	boxed	apples from	Washington.	Calculated	on the
		basis	of all	cars inspecte	ed		

Crop year	Total cars	Percentage of cars show- ing scald	Average per- centage of scald	Average per- centage of blue mold
1922	3,515	16.4	2.8	1.8
1923	2,781	5.3	0.7	0.9
1924	1,713	1.8	0.3	1.7
1925	1,973	2.2	0.2	1.2
1926	1,903	1.9	0.2	1.5
1927	1,825	3.4	0.3	1.8
1928	1,694	3.7	0.4	1.6
1929	2,092	1.9	0.2	2.0
1930	3,811	2.0	0.2	0.6

inspected, the percentage of scald in 1922 was 2.8; in 1923 it fell to 0.7; and during the succeeding years it has maintained an average of less than 0.3 per cent, or approximately $\frac{1}{10}$ the amount for 1922. These percentages are striking, but they do not show the actual effect of the control measure, since the number of cars showing scald had decreased so markedly as to reduce the total number of cars inspected.

The same source of error occurs in the calculation of the percentage of cars that show scald, although this gives a more accurate picture. As indicated in column 3 of table 1 and in the graph, figure 1, the percentage

TABLE 2.—Scald in boxed apples from Washington

Crop year	Total cars inspected that showed dis- ease of any kind	Average percentage of scald calculated on the basis of all cars show- ing diseases of any kind	Average percentage of scald in cars that showed this disease
1917–1920	3,095	5.2	
1922	2,002	4.9	17.0
1923	852	2.4	14.0
1924	693	0.7	14.7
1925	636	0.7	10.5
1926	757	0.6	11.5
1927	842	0.6	7.4
1928	743	0.8	9.5
1929	874	0.4	8.8
1930	997	0.8	10.5

of inspected cars showing scald was 16.4 in 1922, fell to 5.3 in 1923, and in the succeeding years has averaged approximately 2.5, which is somewhat less than $\frac{1}{6}$ of the amount for 1922.

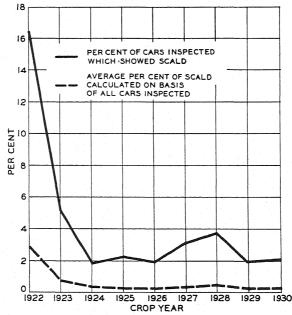


Fig. 1. Amount of scald in boxed apples from the State of Washington as shown by the inspection certificates of the Food Products Inspection Service, Bureau of Agricultural Economics, U. S. Department of Agriculture.

That the crop of 1922 was not characterized by an abnormally large amount of scald is shown in table 2, in which the average amount of scald for the period 1917 to 1920, as calculated by Rose,⁴ is compared with that of the succeeding years. For the purpose of direct comparison with Rose's figures, the percentage is here calculated on the basis of cars that showed disease of any kind. Column 3, table 2, shows that the amount of scald for 1922 was slightly below the average for the years 1917 to 1920. 1923 shows a reduction to somewhat less than $\frac{1}{2}$ of the previous amount, and the succeeding years have continued to show a very small amount of this disease.

In the last column of table 2 is indicated the amount of scald in the cars that showed this disease. Some decrease after the introduction of the treatment is evident, but, as would be expected, the greatest reduction is shown in the number of cars in which scald developed.

LOSSES FROM BLUE MOLD COMPARED WITH SCALD

In order to eliminate the possibility that general conditions had changed so markedly as to affect the general quality of the shipped apples, wholly independent of this special treatment, a computation was made of the losses from blue mold during the same period. Although there is, of course, some variation in the losses from blue mold, calculated on the basis of all cars inspected, there is no evidence of any change similar to that shown for scald. The losses were, in general, somewhat less than those from scald before the control method was developed but very much larger than those from scald under the later conditions.

It is not to be supposed that the figures taken from the inspection sheets and here summarized give a complete picture of the amount of scald in Washington apples throughout the period under review, but these inspections based on over 20,000 cars, made by trained men in central markets throughout the country, must have some significance. It is probably not too much to claim that they furnish the best obtainable indication of the commercial conditions.

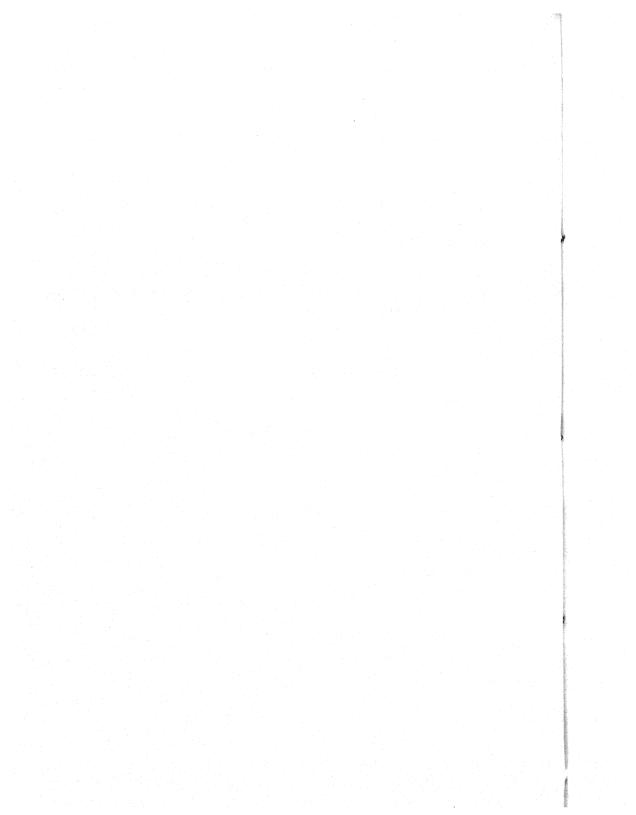
Perhaps the most significant fact brought out by this summary is that the number of inspections requested fell in 1924, after oiled wraps became generally used, to 50 per cent of the number requested in 1922. Although the inspection service continued in popularity and in the confidence of the trade, the number of inspections requested on Washington apples did not for several years approximate the number requested in 1923 and even as late as 1929 was less than 60 per cent of that in 1922.

⁴ Rose, D. H. Diseases of apples on the market. U. S. Dept. Agr. Bul. 1253.

COMMERCIAL IMPORTANCE

It is probably unnecessary to call attention to the fact that the figures presented in this summary, while striking, are by no means an indication of the commercial importance of the control method. An attempt to evaluate the actual significance of this single change in commercial practice would necessitate the consideration of many interesting but highly speculative factors, among which would be an attempt to determine the relative value and salability of lots of apples having less than 1 per cent scald and those having over 5 per cent, the effect on the general apple and fruit market of the presence of numerous cars of badly scalded fruit, and the relation of other diseases to the presence of scald.

BUREAU OF PLANT INDUSTRY, WASHINGTON, D. C.



SOME DISEASES OF WILD POTATOES IN MEXICO

DONALD REDDICK

The discovery that Solanum demissum Lindl. is immune from the blight caused by Phytophthora infestans has directed attention to Mexico as a possible source of breeding stock for a permanent improvement of the potato through the production of disease-resistant sorts. When Humboldt1 explored in Mexico at the beginning of the last century he announced that he found no potatoes in the Republic. His statement has been quoted repeatedly until very recent times. In the meantime, however, about 30 species of tuberous Solani have been described from Mexico many of them endemic to that country. There is very little evidence that Mexican species have entered into the production of the cultivated plant known as S. tuberosum. The possibility that S. demissum blood may be expressing itself at the present time in certain German varieties through the hybrids made by Klotzsch has been suggested elsewhere. 2 Solanum verrucosum, another Mexican endemophyte, was grown for a short time in Switzerland but was discarded because of its susceptibility to blight, apparently without making any impression on the cultivated sorts.

There is little evidence that potatoes ever have played an important rôle in Mexico. The important food sources seem to have been maize (Zea) and beans (Phaseolus). These crops can now be found at all levels, in Mexico, from the sea to an elevation of 10,000 ft. Certainly there is nothing in Mexican archeology comparable to the unmistakable pottery tubers of the Peruvian indigenes. The Spaniards in Mexico unquestionably uprooted many wild potatoes when they constructed walls for their churches and monasteries. Wild potatoes of at least 2 different species can be found now about the monastery at El Desierto, a structure erected fairly early in the 16th century. These plants occur in the ruins of the wall, the church itself, and far up the mountains in deep humic soil under coniferous trees. But the conquistadores were interested in other kinds of nuggets; and in saving souls. There seems to be no evidence whatever that these nuggets

¹ Humboldt, Al. de. Essai politique sur le royaume de la Nouvelle-Espagne. 5 v. F. Schoell, Paris. 1811. Citation in v. 3, pp. 107-108.

In the English translation by John Black (Political essay on the kingdom of New Spain. 2 v. I. Riley, New York. 1811) the statement will be found in v. 2, p. 342.

² Reddick, Donald. Frost-tolerant and blight-resistant potatoes. Phytopath. 20: 987-991. 1930.

of starch were taken back to Spain and certainly nothing in present-day varieties to suggest such a thing.

In October, November, and December, 1930, an effort was made by the Division of Foreign Plant Introduction, U. S. Department of Agriculture, to assemble living material of as many as possible of the Mexican species of tuber-bearing Solani. No attempt was made to secure Solanum fendleri or jamesii from Mexico but, aside from these species, all of the places in Mexico from which wild potatoes have been reported were visited and, in all, perhaps a dozen species were secured. Wild plants were seen in a variety of conditions and all were scrutinized for the presence of disease.

RUST

Rust was found repeatedly on Solanum demissum at El Desierto des Leones, now a national preserve about 40 km. from Mexico City, but was never found elsewhere. When first encountered in October, 1930, the spots were just forming and it appeared that aecia were about to break forth. In a note to J. C. Arthur, the unfortunate mistake of calling the pustules aecia brought a prompt response from him that the fungus was Puccinia pittieriana P. Henn., a short-cycle form, but that "even good mycologists had made the same mistake." This extends the range of the rust considerably farther north than was previously known. The occurrence of rust at El Desierto is well known to such men as Professor Herrera in Mexico City, but no published record is known to the writer and, since he did not mention it, probably none is known to Doctor Arthur. In some cases the rust was very abundant and was doing obvious damage to the plants.

BLIGHT

Typical potato blight was found on some plants of Solanum antipoviczii growing in a recent "fill" along the roadside at 59 km. from Mexico City on the improved road to Cuernavaca. The diagnosis was confirmed by microscopic examination. No record is known to the writer of the occurrence of Phytophthora infestans on wild plants in Mexico. Professor Herrera felt sure that a record existed, but, when he turned to the place where he expected to find it, was unable to do so. The occurrence of this organism in Mexico makes it understandable how it can be that there are growing in the highlands several tuberous species either immune from or very highly resistant to blight. Solanum verrucosum was sent to Switzerland shortly after the outbreak of blight in Europe and was at first considered free from "the disease," but, in a year of severe blight, went down like the local varieties. The species possesses a certain amount of resistance

when compared to the varieties that are commonly grown in North America, but, of all the species in Mexico, it is one of the few that could not give relief from the disease.

Blight was not seen on cultivated plants during the 10 weeks in Mexico for the very good reason that no cultivated plants were seen. Some potatoes are produced throughout this area and were seen in the markets. The 2 common varieties encountered had the appearance of very small Cobbler and Triumph, with the red variety predominating. These commercial sorts, when tested at Ithaca, proved to be susceptible.

SPOT

The most interesting disease encountered on wild potatoes is one that is here called spot. The lesions occur abundantly on the foliage, and clumps of Solanum demissum were found at El Desierto that were nearly defoliated on this account. The very young spot has the glassy appearance of a young lesion caused by Phytophthora infestans. As it enlarges the spot has the appearance of the blight caused by Alternaria solani, but the characteristic target lines are absent. A good many spots may occur on 1 leaf, the intervening tissue turns yellow, and the whole leaf dies, but it usually clings to the stem by a shred. In this condition the plant exhibits the usual symptoms of late blight. This condition has been noted previously on S. demissum when grown in the greenhouse at Ithaca, and it has been suggested elsewhere that this is the disease Lindley and later Klotzsch called "the disease," namely, blight. Spot has appeared abundantly on S. demissum and some other Mexican species when grown recently at Ithaca. The most surprising thing about the disease is the fact that no organism can be found in the tissue. Microscopic examination reveals nothing, and platings The parent plant may show severe spotting and the shoots remain sterile. that come up from long stolons in the same pot may be entirely free. For the present, spot must be considered of physiological nature the cause of which is not known.

VIRUS DISEASES

The relatively cool climate of the *tierra fria* should make it possible to detect such diseases as mosaic on the wild plants at any time. No positive cases of mosaic were encountered. When tubers from the collections were planted at Ithaca, 2 wild plants and 3 plants of cultivated varieties gave indications of mild mosaic. An attempt to transmit the disease to susceptible varieties failed but needs to be tried again.

Leaf roll was not seen in the field and nothing resembling leaf roll or ³ Reddick, Donald. Blight-resistant potatoes. Phytopath. 18: 483-502. 1928.

any of the other virus diseases appeared in the greenhouse cultures. Just how these diseases appear on wild species must await the results of reciprocal inoculation tests—wild plants to healthy cultivated varieties of known reaction; diseased cultivated to wild plants.

TUBER DISEASES

No diseased tubers were found on wild plants, but some tubers in the market were seen that seemed to have the sclerotia of Rhizoctonia on them. Cornell University.

ITHACA, N. Y.

PHYTOPATHOLOGICAL NOTE

Macrosporium solani on tomato fruit.—Rosenbaum showed in 1920 that "nailhead spot" was due to a fungus that had smaller spores than Macrosporium solani and that produced no wine-red coloration in agar cultures, and he ascribed this fungus to the species M. tomato. Nevertheless, in several subsequent publications nailhead spot has been attributed to M. solani.

Macrosporium solani is becoming common in tomato greenhouses in South Australia but has never been found causing a nailhead spot. The fungus can be exceedingly severe on the leaves without causing any injury to the fruit. Figure 1, however, shows the symptoms that were observed in one case of fruit injury that came under notice. The grower stated that "individual tomatoes in a bunch seemed to get it, leaving the remainder of the bunch untouched. The diseased ones were very noticeable as they started to color long before the rest of the bunch."

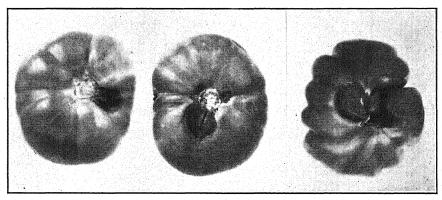


Fig. 1. Lesions caused by *Macrosporium solani* on the stem and the calyx end of Early Dwarf Red tomato fruit.

Most of the infections started either at the stem end or at the calyx end while the fruit was still green, but in a few cases the spots were lateral. The lesions were rather hard, sunken, and slowly spreading on green fruit, but when specimens were kept in a moist dish for about a fortnight the mycelium would spread slowly through the ripening fruit, causing a soft rot with dark brown discoloration.

McWhorter,¹ who had considerable material under observation, figures the black stem-end lesions produced by the fungus on the fruit, stating that it leads to dropping of the fruit, but he makes no mention of nailhead

¹ McWhorter, F. P. The early-blight diseases of tomato. Virginia Truck Exp. Sta. Bul. 59. 1927.

spot as a symptom. It would appear probable, therefore, that the old idea that *Macrosporium solani* can cause nailhead spots on tomato fruit is definitely erroneous.—Geoffrey Samuel, Waite Agricultural Research Institute, University of Adelaide, South Australia.

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THE EFFECT OF DIFFERENT TEMPERATURES ON THE REACTION OF HOPE WHEAT TO BUNT¹

WILLIAM K. SMITH2

In a recent paper (17), reference was made to the reaction to bunt of spring-sown and fall-sown Hope (C. I. 8178),³ a spring variety of *Triticum vulgare* Vill. Because of the possible bearing on the nature of resistance of wheat to bunt, further tests were made on this variety.

Hope has shown consistently a high resistance to various collections of bunt in all spring-sown tests in the hard-red-spring-wheat region of the United States (2, 10, 13). At the Washington Agricultural Experiment Station at Pullman, it has been highly resistant to all collections of the fungus when sown in spring. In the fall of 1928, however, 5 series of 9 winter wheats were inoculated, each series with 1 of 3 physiologic forms of Tilletia tritici (Bjerk.) Wint. and 2 forms of T. levis Kühn, and planted in the experimental field. Hope was included with the 9 winter wheats in each of the 5 series. A similar set of 5 series of spring wheats, including Hope, was planted in the spring of 1929. The reaction of Hope to the forms of bunt in the 2 tests, along with that of susceptible checks, is shown in table 1. Martin has been included because it differentiates the 3 forms of T. tritici and the 2 forms of T. levis.

In table 1, the susceptibility of Hope wheat when planted in the fall is in marked contrast with the strong resistance when planted in the spring. A similar situation was observed by Gaines (5) in Marquis, one of the parents of the Hope variety. He suggests that the resistance of Marquis is neutralized by the lowered temperature or the winter rest period. Other spring wheats, such as Florence (C. I. 4740) and Golden Ball (C. I. 6227), which are resistant to Tl and have been tested from fall and spring planting with this form, have been markedly resistant in all tests at the Washington Agricultural Experiment Station.

A comparison of the reactions of fall-sown Hope and Hybrid 128 shows that Hope has, with all 5 forms, lower percentages of bunt than Hybrid

- ¹ Published as Scientific Paper No. 213, College of Agriculture and Experiment Station, State College of Washington.
- ² The writer is indebted to Dr. E. F. Gaines for the materials used and to Dr. H. F. Clements for advice and criticism in this study.
- ³ Accession number of the Division of Cereal Crops and Diseases, U. S. Department of Agriculture.

TABLE 1.—The reaction of Hope wheat to physiologic forms of bunt when planted in the fall of 1928 and the spring of 1929. Martin differentiates the 3 forms of Tilletia tritici and the 2 forms of T. levis

	Date						
Variety	of planting	T1a	T2	тз	L4a	L5	
Martin	1928 Oct. 9	0	19	71	51	0	
Hope	"	51	60	42	61	78	
Hybrid 128	"	68	83	90	84	90	
Hope	1929 Apr. 24	0 92	0 78	0 73	0 58	0 56	

^a T and L denote physiologic forms of T. tritici and T. levis, respectively.

128. Other trials involving the 2 varieties indicate that Hope, when sown at this season, is not completely susceptible. It is, however, apparent that there is a striking difference in Hope between the percentages of bunt in the plantings made on the 2 dates listed in table 1. The greatest contrast between the environmental conditions prevailing in the 2 plantings lies in the trend of temperatures subsequent to the emergence of the seedlings from the soil; in the fall the mean daily temperatures gradually become lower, reaching a minimum towards midwinter, while in the spring they tend to rise rather rapidly. Accordingly, an attempt was made to obtain further information on the relation of the temperature after emergence to the incidence of bunt in this variety.

WORK OF PREVIOUS INVESTIGATORS

Of the many environmental factors influencing the amount of bunt in any variety, temperature is one of the most important. All the available evidence indicates that *Tilletia levis* and *T. tritici* are similar in their reaction to temperature.

Earlier work on the relationship between temperature and infection has been reviewed adequately by Woolman and Humphrey (21) and Caspar (3). It is sufficient here to note that, although the earlier investigators were not in agreement as to the optimum temperature for infection, the situation has now been clarified by studies on the temperature range for the germination of wheat seeds and bunt spores. The data given by Hecke (8), listed below, are in general agreement with those of other workers.

A comparison of the data on the germination of wheat seeds and bunt spores indicates that a certain temperature would be optimum for the in-

Germination temperatures

	Wheat	Bunt spores
Minimum	3–4.5° C.	slightly less than 5° C.
Optimum	25° C.	16–18° C.
Maximum	30–32° C.	less than 25° C.

fection of inoculated wheat, while, with temperatures rising or falling from this point, there would be a decrease in the amount of bunt. This has been confirmed by Munerati (15), who obtained no bunt in inoculated wheat grown at a temperature ranging from 2 to 4° C. and none in inoculated wheat at 22 to 25° C., although plants grown at intermediate temperatures were heavily smutted. Heald and Woolman (7) made a series of sowings of bunt-inoculated seed under field conditions from August 24 to November 23 in 1914 and obtained low percentages of bunt when either high or low temperatures prevailed during the germinating period, while at intermediate temperatures high percentages were recorded. Although it must be borne in mind that in this test, as in other experiments on temperature relations under field conditions, other disturbing environmental factors play a part, e.g., moisture, the results are in agreement with the deductions drawn from the above-mentioned data of Hecke. Similar results have been obtained by other investigators.

There is general agreement with the findings of Wolff (19) that the wheat plant is infected usually between the time of germination and that of emergence of the first green leaf from the coleoptile. Woolman and Humphrey (22), however, in 1 experiment transferred to inoculated soil seedlings that had been grown in sterile soil and that showed 1.25 to 1.5 in. of green leaf and obtained 2 bunted plants from a total of 52. Transplanting would, however, cause considerable root injury and would check the normal development of the plants. Milan (14) succeeded in infecting wheat plants at any time during their growing period by pricking the base of the culms with a needle or by breaking off the culms near the roots and applying germinating spores of Tilletia levis and T. tritici. Bunted plants were also obtained by Bodine and Durrell (1) by injecting into the base of the culms or inserting inside the sheath cultures of T. levis.

The influence of environmental conditions prevailing after the emergence of the seedling from the soil or of the first green leaf from the coleoptile has received meager study. Hecke (8) points out that the entrance of the fungus into the tissues of the plant marks only the beginning of the fight between host and parasite and suggests that temperature may affect this reaction by influencing (a) the germination of spores and seeds, (b) the length of the infection stage in young plants, and (c) the possibility of the fungus reaching the growing point.

Attention was called by Faris (4) to the possible influence of growth conditions subsequent to germination on the development of bunt. Two varieties of winter wheat were inoculated with *Tilletia levis* and *T. tritici* and germinated in temperature tanks in the greenhouse. One-half of the seedlings of each variety were then planted in the field and the other half in the greenhouse. There was no evidence that the growth of the host after germination had a marked effect on the development of bunt in the 2 varieties.

Caspar (3) made observations on wheat grown in the shade of fruit trees and in the open to determine the effect of shade on bunt reaction. The presence of more than one variable factor renders the results inconclusive. Rabien (16) suggests that, since partly smutted plants occur, the environment after the germinating period may affect the reaction of plants to bunt.

It is apparent that little consideration has been given to the effect of environmental conditions after the emergence of the seedlings from the soil. On the contrary, it has been generally assumed that there is no influence after this stage of development. For example, in a discussion of equipment and methods of studying the relation of soil temperature to disease in plants, Leukel (12) states: "As infection by many of the cereal diseases such as bunt, for example, occurs only during the seedling stage of the plant, it is not always necessary to grow the plants at different temperatures beyond the stage of the fifth, fourth, or even the first leaf." Although, under normal conditions, infection may not take place after the appearance of the first green leaf, the reaction between host and parasite after this stage in infected plants of certain varieties may not be the same at different temperatures.

METHODS AND EXPERIMENTAL RESULTS

Field experiments. The data presented in table 1 show that Hope, when planted on October 9, 1928, was susceptible to 5 physiologic forms, while a planting made on April 24, 1929, was smut-free or strongly resistant to the same forms. During the fall of 1929 and the spring of 1930, plantings of Hope were made at different dates to find the reaction to bunt when seeded under conditions different from those of the usual dates of fall and spring sowing. Jenkin (C. I. 5177), a spring variety of Triticum compactum Host, was used as a control. Unfortunately, fall rains did not commence until late in the season, so that the first planting was not made until October 29. Owing to the low moisture content and low temperature of the soil after this date, the seedlings grew slowly and, in the later plantings, had not appeared aboveground when the soil was frozen and the plot covered with its winter blanket of snow. At each date, 3 rod rows of each

variety were sown, the seeds being first inoculated with T2 and planted at the rate of 75 seeds per row. The dates of planting and percentages of bunt are shown in table 2.

TABLE 2.—Percentages of bunt in Hope and Jenkin, inoculated with physiologic form T2 and sown at different dates

Date of	37		Average		
of Variety planting	Row 1	Row 2	Row 3	Average	
1929 Oct. 29	HopeJenkin	Per ct. 48 91	Per ct. 40 97	Per ct. 49 98	Per ct. 45.7 95.3
Nov. 5	Hope	26 67	25 53	28 71	26.3 63.7
" 12	Hope Jenkin	8 48	17 55	10 41	11.7 48.0
" 19	Hope Jenkin	4 6	$\begin{array}{c} 1 \\ 12 \end{array}$	0.7 8	1.9 8.7
" 26	Hope Jenkin	2 5	6 5	4 14	4.0 8.0
1930 Mar. 14	Hope Jenkin	5 72	11 71	5 61	7.0 68.0
" 29	Hope Jenkin	3 67	2 63	2 57	$\frac{2.3}{62.3}$
Apr. 5	HopeJenkin	3 73	4 71	3 66	3.3 70.0
7	Hope Jenkin	0 79			
1929 Apr. 24	Hope Jenkin	0 78			
1931 Apr. 11	Hope	0.6 66		1	

In table 2 the amount of bunt in Hope in the planting made on October 29 is quite similar to that recorded in table 1 for normal sowing time in the fall. In the plantings made later in the fall, the percentage of bunt decreases in each of the 2 varieties, the reaction of Hope paralleling that of Jenkin. In the spring, owing to the high moisture content of the soil, seeding could not be done earlier than March 14. In the planting made on this date, however, the amount of bunt in Hope is relatively higher than in the

later spring plantings and in those made at the usual time for spring wheat in other years in which the strong resistance of Hope and high susceptibility of Jenkin are apparent. It is probable that if environmental conditions would permit sowing earlier than March 14, the percentage of bunt in Hope would be higher than the percentages recorded for this variety in the spring of 1930.

GREENHOUSE EXPERIMENTS

Reaction to physiologic form T2. The unsatisfactory nature of field experiments in testing the effect of temperature has been noted previously. Accordingly, in the winter of 1929–30, a test was made in the greenhouse to study further the influence of temperature. Hope and Jenkin were again used. The 2 varieties were planted in 4 flats (17 in. × 34 in. × 3½ in.) in alternate rows, the seeds inoculated with T2 being planted 2 in. apart in rows 2 in. apart and at a depth of 2 in. The soil used was a mixture of 3 parts of Palouse loam to 1 part of sand. The boxes were watered at intervals to maintain what was considered the soil-moisture optimum for the development of the fungus; daylight was supplemented from sunset until 1:00 a. m. by a 100-watt Mazda lamp at a distance of approximately 30 in. directly above each box. The material was grown in 3 sections of a greenhouse in which temperatures were maintained by the manipulation of steam valves and air vents; 1 section was kept warm (approximately 21° C.), another cool (approximately 9° C.), and the third intermediate (approxi-

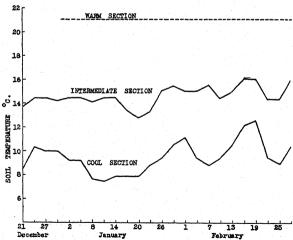


FIG. 1. Average soil temperatures in 3 sections of the greenhouse during a test of the reaction of Hope and Jenkin to a physiologic form of bunt (T2). The curves for the cool and intermediate sections were taken from soil-thermograph records, but the curve for the warm section represents the mean of thermometer readings taken at intervals during each day.

mately 15° C.). Because of the absence of thermostats, there were daily fluctuations of temperature in each section. The variations were brought about by external climatic conditions, so that all sections were influenced in a similar manner. The temperature in the cool and intermediate sections, respectively, was recorded by soil thermographs, while that in the warm section was read at intervals. The trend of temperatures is shown in figure 1, in which the mean temperatures of 3-day periods from December 20 until March 1 are diagrammed.

In figure 1, considerable fluctuation in mean temperature is observed, but the sections in which a continuous record of soil temperature was available show curves that are approximately parallel.

The 2 varieties Hope and Jenkin were planted on December 18 in 4 flats that were treated subsequently as indicated in table 3.

TABLE 3.—Effect of different temperatures on Hope and Jenkin grown from seeds inoculated with T2 and planted in the greenhouse December 18, 1929

Mammanatura		No. 0	f plants		Wt. per plant,	Period from planting to heading	
Temperature during growth	Variety	Bunted	Bunt- free	Bunt	exclud- ing roots		
				Per ct.	Gm.	Days	
Low continuously	Hope Jenkin	45 39	$egin{array}{c} 0 \ 4 \end{array}$	100 90.7	.51 .74	94.3 ± .44 105.6 ± .38	
Low until emergence from soil, then intermediate	Hope Jenkin	18 35	17 2	51.4 94.6	.26 .31	84.4 ± .54 88.2 ± .56	
Intermediate continuously	Hope Jenkin	17 33	13 1	56.7 97.1	.27 .37	84.1 ± .74 86.6 ± .59	
Low until emergence from soil, then high	Hope Jenkin	1 37	40 4	2.4 90.2	.18	81a 80a	

^a The average number of days from planting to heading in this treatment was computed from counts of headed plants taken at 5-day intervals, whereas in the other treatments the plants, of which the spike had emerged from the sheath, were tagged individually at 2-day intervals.

In table 3 it is apparent that, while Jenkin has a high percentage of bunt at all 4 temperatures, striking differences are present in the reactions of Hope: the amount of bunt in Hope ranges from 100 per cent in the cool section to 2.4 per cent in the flat kept at the low temperature until the emergence of the seedlings from the soil and then transferred to the warm section. It will be noted from the average weight per plant (air-dried) that the plants growing at the lower temperatures were more vigorous than the others. Those grown from emergence to maturity in the warm section

were somewhat stunted in growth, and 25 of the 40 bunt-free plants of Hope were sterile; of the 4 bunt-free plants of Jenkin, 2 were quite sterile and the other 2 produced a few seeds, the remainder of the flowers being sterile. The smutted plants of either Jenkin or Hope in this section showed only rarely any sterile flowers, *i.e.*, if an ovary develops on a spike invaded by the fungus, a smut ball apparently will result. Further, microscopic examination of ovaries from the sterile plants of Hope, treated with suitable stains, did not reveal any trace of the fungus. The abovementioned 25 sterile plants of Hope and 2 plants of Jenkin may be regarded as smut-free, even though no seeds were produced.

The most striking divergence in the reaction of Hope is the difference between the plants kept continuously at the low temperature and those transferred after emergence to the relatively high temperature. All other important environmental factors, such as moisture and light, were kept as uniform as possible throughout the 3 sections. It may, therefore, be concluded that this difference in the reaction of Hope is due to the difference in temperature after the seedlings appeared aboveground.

It is of some interest to compare the reaction of Hope in the other 2 treatments. Although in the one the plants were kept at a low temperature during what is regarded as the infection stage, the resulting amount of bunt is approximately the same in both, while in the treatment in which the temperature was low continuously the percentage of bunt is much greater. In these comparisons, the controlling action of the temperature after emergence is demonstrated for this variety.

Nature of the reaction. What then are the factors responsible for this reaction in Hope? It has been observed frequently that a susceptible wheat, inoculated at sowing time and germinated at relatively high temperatures, produces at maturity a normal yield of seed and no smut balls. This phenomenon has been explained by assuming that, because of the difference in optimum temperature for the growth of host and parasite, the fungus grows more slowly than the wheat plant and fails to reach the growing points of the shoots. If such were the case in this variety, it might be expected that, at the higher temperatures, Hope would give some evidence of more rapid development than Jenkin. The number of days from planting to heading, presented in table 3, does not support this explanation. It is recognized that this criterion of speed of growth may not be very significant because growth of the culms after the tillering stage takes place mainly through nodal rather than terminal growth; if the fungus reaches the young spike during the tillering period, it will be carried up in the spike as the elongation of the culms takes place. Examination of the data shows that the rate of growth in these varieties, as measured by the time from planting to the emergence of the spike from the sheath, is the reverse of what might be expected: at the low temperature Hope headed out $11.3 \pm .6$ days earlier than Jenkin, while at the higher temperature the 2 varieties headed at approximately the same time. Heuser (9), however, points out that one variety may grow more rapidly than other varieties at one stage of growth but more slowly than others at other stages of growth. He cites Criewerer 104, a resistant wheat, which emerged from the coleoptile in a shorter period than other wheats but which headed and ripened later than the others.

In order to test the comparative speed of growth of the 2 varieties in the earlier stages at different temperatures, a test was made in the spring of 1930 similar to that of the previous year. Four flats were again used but the seeds were planted 1 in. apart in the row instead of 2 in. Planting was made on March 16.

It was observed that in all 4 flats the Hope seedlings emerged from the soil somewhat earlier than those of Jenkin. When in any flat the first green leaves had attained an average length of 6 to 7 cm., a measurement was made from the tip of the leaf to the tip of the coleoptile on all seedlings in the flat. The data are summarized in table 4.

TABLE 4.—Comparative length of the first green leaf measured from the tip of the leaf to the tip of the coleoptile in Hope and Jenkin grown at different temperatures; in each treatment, Jenkin has been represented as 100

Treatment		Length of the first green leaf			
No.	Temperature during growth	Jenkin	Норе		
1	Low continuously	100 ± 1.79	126.4 ± 3.07		
2	Low until emergence, then intermediate	100 ± 1.64	124.4 ± 2.45		
3	Intermediate continuously	100 ± 1.84	123.9 ± 2.75		
4	Low until emergence, then high	100 ± 1.65	118.0 ± 2.14		

It is apparent in table 4 that, assuming the growth of the first green leaf to be a criterion of rate of development, Hope grew more rapidly than Jenkin in all 4 flats. Since Jenkin has been represented as 100 in each of the 4 treatments, the growth rates of Hope may be readily compared. The important comparison, however, is between No. 1 and No. 4. If the resistance of Hope in treatment 4 is due to the host outgrowing the fungus at the higher temperature, Hope would be expected to have a greater growth relative to Jenkin in No. 4 than in No. 1. This, however, is not the case. On the contrary, the growth of No. 1 exceeds that of No. 4 by 8.4 ± 3.7 . Although this difference is scarcely significant, the data show clearly that the growth of Hope relative to that of Jenkin is not higher when trans-

ferred to high temperature after emergence than it is when low temperature is maintained. Therefore, the high resistance of Hope when transferred to the warm section cannot be attributed to more rapid growth at this stage of development.

DISCUSSION

The results presented in table 3 show that Hope, when grown until emergence from the soil at a temperature considered to be near the optimum for infection and subsequently transferred to a temperature of approximately 21° C., is highly resistant to the T2 physiologic form of bunt. Under field conditions the reaction of Hope to form T2 is almost identical with its reaction to the other 4 forms listed in table 1. It has been shown (17) that the factors for resistance against any 1 of the forms are the same as those for resistance against any of the other 4. It is, therefore, highly probable that the reaction of Hope to these forms at different temperatures in the greenhouse would be the same as the reaction to T2 in this experiment. The striking differences in the amount of bunt in fallsown and spring-sown plants of this variety under field conditions seem to be due to the fact that in spring planting there is a tendency towards higher temperatures soon after the seedlings emerge from the soil, while in fall planting this rise in temperature does not take place until after an interval of several months.

How, then, is the development of the fungus checked at the higher temperatures? Woolman (20) has shown that at least 1 physiologic form of *Tilletia tritici*, probably identical with T1, enters the tissues of certain resistant and susceptible wheats alike; and there is no evidence that other forms behave differently. We may therefore accept the conclusion of Hecke (8) that the fight between host and parasite only begins after the entry of the fungus into the tissues of the plant.

A number of investigators in Germany and Austria, notably von Tubeuf, Appel and Gassner, von Kirchner, Hecke, and Heuser, have studied the relationship between speed of germination and reaction to bunt. Conflicting results have been obtained; some workers found a distinct relationship, while others found that their results did not allow them to generalize. In the recent investigations of Straib (18), only a low correlation was apparent in 7 spring wheats between reaction to bunt and speed of germination as determined from tests in the seed laboratory. A fairly high negative correlation, however, was found between the amount of bunt in 8 varieties of spring wheat and the average length of the first green leaf a few days after its emergence from the coleoptile. In the present experiment, however, the results indicate that the striking resistance of Hope at the higher temperatures is not due to a relatively greater speed

of growth as determined by growth of the first green leaf or time of heading.

There are numerous references to the relationship between cell-sap acidity and disease resistance in plants, but, as Hurd-Karrer (11) points out, the literature on the question "indicates a wide-spread belief that these are related characters, but leaves the impression that the data on which positive claims are based are too often meager or unconvincing." In 6 resistant and 5 susceptible wheats, Hurd-Karrer found no consistent relationship between resistance to Tilletia tritici and titratable acidity or hydrogen-ion concentration. Giljarovskij and Zak (6), however, in a recent study of a number of common and durum wheats report that resistance to T. tritici is correlated with energy of growth, together with the change of hydrogen-ion concentration towards the alkaline side and the rise in osmotic pressure.

In recent years, with the increasing number of demonstrations of physiologic forms within species of parasitic organisms, the limited application of the concept of a relationship between gross plant characters and reaction to disease has become increasingly apparent; a host variety may be immune from one physiologic form and quite susceptible to another under the same environmental conditions. The above-mentioned concept may apply to the present example, since in table 1 Hope is equally resistant to all forms when spring sown and susceptible to all forms when fall sown. It is, therefore, possible that at one temperature the internal composition of the variety is favorable for fungal growth, while at other temperatures it may not be suitable. No attempt has been made to obtain information on this point. It is also possible that, in the reaction of Hope to bunt, there is an organization of the protoplasm at the low temperature that permits the development of the fungus, while at the higher temperature the organization inhibits or retards fungal growth.

SUMMARY

Hope, a variety of *Triticum vulgare*, has been highly resistant to 3 physiologic forms of *Tilletia tritici* and 2 forms of *T. levis* when planted at the time usual for spring sowing but has been moderately susceptible to all 5 forms when planted at the usual sowing time in the fall.

Plantings of Hope and Jenkin, a susceptible variety of *Triticum compactum*, were made in the field late in the fall of 1929; the seeds were inoculated with a physiologic form of *Tilletia tritici* (T2) and planted at weekly intervals from October 29 to November 26. The percentage of bunt decreased in both varieties, the reaction of Hope paralleling that of Jenkin.

In the plantings made early in the spring of 1930 Hope was resistant

and Jenkin was susceptible, but in the early plantings Hope was less resistant than when sown at the normal date for spring grains.

The relation between temperature and reaction to bunt at different stages of growth was tested for Hope and Jenkin with seeds inoculated with T2 and planted in the greenhouse in the winter of 1929–30. Hope was resistant when grown at a relatively low temperature until emergence from the soil and then grown at a higher temperature, although plants grown continuously in the cool environment were quite susceptible. Jenkin was quite susceptible under conditions of both low and high temperature.

The different reactions exhibited by Hope in fall and in spring plantings seem to be due mainly to the respective temperatures subsequent to the emergence of the seedlings from the soil.

The relative growth rates of Hope and Jenkin after emergence from the soil showed that Hope is not resistant at the higher temperature by reason of more rapid development as measured by rate of growth of the first green leaf or time of emergence of the spike from the leaf sheath.

It is suggested that the resistance of Hope at the higher temperature is dependent on either unfavorable nutritional conditions or an organization of the protoplasm that at this temperature retards or inhibits the growth of the fungus.

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A NONINFECTIOUS LEAF-DEFORMING PRINCIPLE FROM MOSAIC TOMATO PLANTS^{1,2}

H. R. KRAYBILL, P. H. BREWER, R. W. SAMSON, AND M. W. GARDNER

By filtration methods Kraybill and Eckerson³ separated the juice from mosaic tomato plants into 2 fractions, the residue that contained the mosaic virus and the filtrate that, when introduced into healthy plants, produced certain of the symptoms of typical mosaic, such as a stunting of the plant and various leaf deformities, including the filiform type but no mottling. It was uncertain whether the leaf-deforming principle of the filtrates was infectious, that is, whether it could be transferred from plant to plant, whether it was formed only in the juice from mosaic-diseased plants, or whether it was formed from the decomposition of some compounds present in healthy as well as diseased plants.

In the studies reported in this paper we have found that the leaf-deforming principle in the filtrates is not infectious and that it also differs from the mosaic virus in that it is not diminished in activity by heating at 126° C. for 2½ hours, a treatment which inactivates the virus. The most active preparations are obtained by permitting the juice of mosaic plants to autolyze or ferment previous to filtration. This suggests the possibility that the active principle of the filtrates may be a decomposition product of the tomato-plant juice and may have no relation to the virus. While negative results are not conclusive proof, in no case were active filtrates obtained from healthy tomato plants.

METHOD OF INOCULATION

Young tomato plants having 3 or 4 leaves developed were used for inoculation. In order to get results with the leaf-deforming principle, it is essential to use young plants and to inoculate heavily. In the process of inoculation the leaf was supported with a piece of tissue toweling held in one hand while a drop of the inoculum was placed on a leaflet by means of a pipette. Then, by means of a sterilized needle, the leaflet was scarified and the drop of inoculum spread on the wounds. Several leaflets of each of 3 or 4 leaves were inoculated on each of 3 successive days.

- ¹ Contribution from the State Chemist and Botany Departments, Purdue University Agricultural Experiment Station, La Fayette, Ind.
- ² Most of the material in this paper was presented before the Twentieth Annual Meeting of The American Phytopathological Society, New York, December 28, 1928, to December 31, 1928. Abstract in Phytopathology, 19: 108. 1929.
- ³ Kraybill, H. R., and S. H. Eckerson. Tomato mosaic. Filtration and inoculation experiments. Amer. Jour. Bot. 14: 487-495. 1927.

PREPARATIONS USED IN INOCULATIONS

- A. A large number of healthy tomato plants were ground in a Nixtamal mill on May 12, 1927. The juice was pressed out in a tincture press. Five hundred cc. of the juice was placed in an Erlenmeyer flask and allowed to ferment for about 2 weeks at room temperature. The material was then filtered through a Jena glass-fritted filter 6/<7. The filtrate was used for inoculations.
- B. Mosaic tomato plants from the greenhouse were ground in a Nixtamal mill on September 6, 1927. The juice was pressed out and 300 cc. was allowed to ferment from September 6 to October 10. Then, the juice was filtered through filter 6/<7. The filtrate was used for inoculation.
- C. Healthy tomato plants taken from the greenhouse on September 6, 1927, were used. Juice was prepared in the same manner as B.
- D. Mosaic-diseased tomato plants from commercial fields were ground in a Nixtamal mill. Fifteen hundred cc. of juice, pressed out by a tincture press, was allowed to ferment from September 9 to 17, 1927. The juice was then filtered through Jena glass-fritted filter 6/< 7. The filtrate was used for inoculation.
- E. Mosaic-diseased plants from the same lot from which D was prepared were ground in a Nixtamal mill. The juice was pressed out in a tincture press and centrifuged in a Sharples supercentrifuge and then stored in the refrigerator.
- F. Mosaic-diseased tomato plants from commercial fields were ground in a Nixtamal mill and the juice pressed out with a tincture press September 23, 1927. About 500 cc. of juice was placed in an Erlenmeyer flask, covered with a layer of toluol, and kept in the ice box from September 23 to October 22. The juice was then filtered through filter 6/< 7 and kept covered with toluol. The filtrate was used for inoculation.

SYMPTOMS PRODUCED BY THE LEAF-DEFORMING PRINCIPLE

Plants inoculated heavily with noninfectious preparations from mosaic plants are temporarily stunted and the young developing leaves show a range of deformation varying from a slight narrowing to an extreme filiform condition of the leaflets, accompanied usually by considerable puckering, curvature, and twisting (Figs. 1 and 2). Portions of the surface of certain of the deformed leaflets may exhibit a dull greyish cast. There is no mottling of the leaflets as in typical mosaic. Furthermore, the leaf-deformity symptoms do not continue to appear on new growth as in mosaic, but the plants soon outgrow the condition and the later-formed leaves are normal.

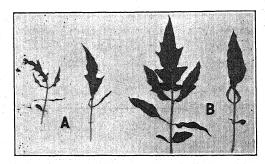


Fig. 1. Leaf deformities (narrowing of leaflets, filiform tendencies, and curvature) produced by heavy inoculation of young tomato plants with leaf-deforming principle (preparation D after 4 years of storage). A. Fifth and sixth leaves of 1 plant. B. Fifth and sixth leaves of another plant comparable as to age and time of inoculation. Later, normal growth was resumed.

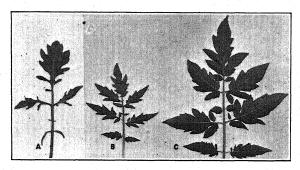


Fig. 2. A. Filiform leaf deformity produced by heavy inoculation of young tomato plant 2 weeks previously with noninfectious preparation D after it had been heated at 96° C. for 10 minutes. B. Dwarfing of the leaf, as compared with C, and slight narrowing of the leaflets produced by inoculation with the leaf-deforming principle. C. Sixth leaf of uninoculated plant of same age as inoculated plant of which B is the 6th leaf.

LEAF DEFORMITIES PRODUCED WITH INFECTIOUS PREPARATION

Marked leaf deformities were produced in 15 plants inoculated with an infectious preparation E, which had been stored in the refrigerator for 4 months, and in all but one of the plants mosaic mottling also occurred (Table 1).

Inoculations made from these plants (Table 2) showed that the mottled plants contained the virus and that the 1 plant showing only leaf deformities did not contain the virus.

Regardless of what viruses may have been present in the original plants from which preparation E was obtained, these results are of especial inter-

est in view of the fact that Mogendorff⁴ was able to produce fern-leaf symptoms only by inoculation with the cucumber-mosaic virus, which will not remain infectious more than 3 days *in vitro*^{5, 6} and, consequently, could not have been present in this inoculum.

TABLE	1.—Inoculations	with	preparations	from	healthy	and	mosaic	tomato	plants

Inoc- ulum	Source of inoculum	Date of inoc.	Number plants inoc.	Number showing no symptoms	Number with leaf deformities only	Number with mosaic symptoms
$\overline{\mathbf{q}}$	Mosaic	12-12-27	15	2	13	0
C	Healthy	12-12-27	15	15	0	0
Water		12-12-27	15	15	0	0
A	(C)	12-16-27	15	15	0	0
\mathbf{F}	Mosaic	12-16-27	15	12	3	0
Water		12-16-27	15	15	0	0
A	Healthy	5-25-27	20	20	0	0
\mathbf{E}	Mosaic	1-13-28	15	0	1	14
Water		1-13-28	15	15	0	0
A	Healthy	7-23-28	15	15	0	0
В	Mosaic	7-23-28	15	11	4	0
$^{\rm C}$	Healthy	7-23-28	15	15	0	0
\mathbf{D}	Mosaic	7-23-28	15	7	8	0
\mathbf{F}		7-23-28	15	13	2	0
Water	11	7-23-28	15	15	0	0
D	•	11-12-31	20	8	12	Ó

LEAF DEFORMITIES PRODUCED WITH NONINFECTIOUS PREPARATIONS

With preparation D (3 months old), 15 plants were heavily inoculated and marked leaf deformities developed in 13 of the plants and none showed any mosaic mottling (Table 1). In a similar test made when this preparation was 10 months old, 8 out of 15 plants developed the leaf deformities 10 days after inoculation, and in another test, made when it was over 4 years old, 12 out of 20 plants inoculated developed leaf deformities.

With preparation F (3 months old), 15 plants were inoculated and 3 developed leaf deformities. In a similar test made when this preparation was 10 months old, 2 out of 15 plants developed the leaf deformities in 10 days.

With preparation B (10 months old), 15 plants were inoculated and 4 developed leaf deformities. Check inoculations with pure water and with

⁴ Mogendorff, N. "Fern-leaf" of tomato. Phytopath. 20: 25-46. 1930.

⁵ Doolittle, S. P. The mosaic diseases of cucurbits. U. S. Dept. Agr. Bul. 879. 1920.

⁶ Johnson, James. The classification of plant viruses. Wis. Agr. Exp. Sta. Res. Bul. 76. 1927.

the juice from healthy plants (preparations A and C) yielded no leaf deformities.

All of these preparations had undergone a short period of fermentation previous to filtration and storage.

PREPARATION FROM JUICE OF HEALTHY PLANTS NOT ACTIVE

Attemps were made to determine whether it is possible to secure preparations from the juice of healthy tomato plants that would produce symptoms similar to those produced by preparations from the juice of mosaic plants. Neither of the preparations A and C from the juice of healthy tomatoes produced leaf deformities when used as inoculum, as shown in table 1. At the time of the inoculations (July, 1928) preparation A was 14 months old and C was 10 months old and both preparations had undergone preliminary periods of fermentation.

Although these negative results cannot be interpreted as definite proof, they indicate that the principle in the filtrates of the juice from mosaic tomato plants has some connection with the fact that the plants are diseased

EFFECTS OF INOCULATION WITH LEAF-DEFORMING PRINCIPLE NOT TRANSFERABLE FROM PLANT TO PLANT

In order to determine whether the leaf-deforming principle is infectious, plants showing the leaf deformities produced by inoculating with the leafdeforming principle were crushed and the juice used to inoculate healthy plants. Ten plants that had been inoculated previously with preparation D and that showed leaf deformities but no typical mottling were selected for the experiment. The individual plants were ground in an iron mortar that had been previously placed in boiling water for 5 min. Fifty cc. of distilled water was added and the extract poured off into a test-tube and preserved by adding a few drops of toluol. The juice from each plant was used to inoculate 5 healthy plants on each of 3 successive days. In no case did the inoculated plants show any symptoms of typical tomato-mosaic or symptoms similar to those produced by the leaf-deforming principle.

In order to check this procedure, 10 tomato plants that had been previously inoculated with juice from mosaic plants (preparation E) were used in the 2nd experiment. Nine of these plants showed typical tomatomosaic symptoms and marked filiform leaf deformities, while 1 (N 6, Table 2) of the plants showed no mottling but showed symptoms similar to those produced by the leaf-deforming principle.

A water extract of the crushed tissues of each of the 10 plants was prepared in the same way as in the previous experiment. The juice prepared from each individual plant was used to inoculate 8 healthy plants.

In all cases (Table 2) the 8 plants inoculated developed typical symptoms of tomato mosaic with the exception of the 8 plants inoculated with the juice from plant N 6, which had shown no mosaic mottling. These 8 plants showed no symptoms whatever.

These results show very definitely that the leaf-deforming principle is not infectious and cannot be transferred from plant to plant in the same manner as the virus of typical tomato mosaic.

TABLE 2.—Inoculations with fresh juice from 9 mosaic plants and 1 $(N \ 6)$ showing only leaf deformities

	Source of inoculum	Number	Number	Number with	
Plant number			normal	typical mosaic symptomsa	
N 1	Typical mosaic and leaf deformities	8	0	8	
\mathbf{N} 2		8	0	8	
N 3		8	0	8	
N 4	66	8	0	8	
N 5	(C)	8	0	8	
N 6	Leaf deformities but no mottling	8	8	0	
N 7	Typical mosaic and leaf deformities	8	0	8	
N 8	•	8	0	8	
N 9		8	0	8	
N 10		8	0	8	

a Certain of the plants showing mosaic symptoms also showed leaf deformities.

LEAF-DEFORMING PRINCIPLE COMBINED WITH POTATO VIRUS FAILS TO PRODUCE STREAK

Further evidence that the leaf-deforming principle is not the tomatomosaic virus was obtained by the failure to produce the streak or double-virus-mosaic disease in any of a set of 10 tomato plants inoculated with the leaf-deforming principle (D) and the virus present in apparently healthy potatoes. Out of a similar set of 10 plants inoculated only with the leaf-deforming principle, 6 developed leaf deformities, and all in another set of 10 inoculated with tomato-mosaic virus and the potato virus developed the streak disease.

Furthermore, the juice from inoculated tomato plants (M 1 to M 10) showing only the leaf deformities, when combined with the potato virus, failed to produce streak, as shown in table 3, whereas the typical streak developed when the tomato-mosaic virus was combined with the potato virus.

TABLE 3.—Results of inoculation with potato virus and juice from plants showing only leaf-deformity symptoms

	First	series	Second series		
Source of inoculum	Number plants	Number streak	Number plants	Number streak	
M 1+Potato	õ	0	15	0	
M 2+ "	6	0	15	0	
M 3+ "	6	0	15	0	
M 4+	6	0	15	0	
M 5+ ''	6	0	15	0	
M 6+ "	6	0	15	0	
M 7 + ''	6	0	15	0	
M 8+ ''	6	0			
M 9 + ''	6	0	15	0	
M 10 + ''	6	0	15	0	
Potato			15	0	
Mosaic tomato + potato	10	10	15	13	

^a M 1 to M 10 are plants showing only leaf deformities.

LEAF-DEFORMING PRINCIPLE HEAT STABLE

The leaf-deforming principle is heat stable. It apparently suffers no loss in activity when heated for $2\frac{1}{2}$ hours at 126° C. in an autoclave. Table 4 shows the results of heating the preparation D at various temperatures, and a leaf from 1 of the subsequently inoculated plants is illustrated in figure 2, A.

TABLE 4.—Effect of temperature on leaf-deforming principle

Temperature Time of ± 0.2° C. heating		Number plants inoc.	Plants showing leaf deformities	Plants showing no leaf deformities	
° C. 80.0	10 min.	10	7	3	
84.0	10 ''	10	10	0	
88.0	10 ''	10	10	0	
92.0	10 ''	10	10	0	
96.0	10 ''	10	9	1	
98.6	10 ''	10	6	4	
126.0	2.5 hrs.	10	10	0	

SUMMARY

Filiform leaf deformities closely resembling certain mosaic symptoms were produced on the new growth of young tomato plants by heavy inoculation with preparations obtained from mosaic plants and rendered non-infectious by filtration. Subsequently normal growth was resumed.

Leaf deformities were not produced by inoculation with similarly prepared juices from healthy tomato plants.

The leaf deformities were readily produced with infectious preparations previously stored *in vitro* so long that the possibility of the presence of active cucumber-mosaic virus was eliminated.

When plants showing marked leaf deformities as a result of inoculation with the leaf-deforming principle were crushed and used as inoculum for healthy plants, no leaf deformities resulted. This indicates that the leaf-deforming principle is not transmissible from plant to plant as is a virus, at least in quantities sufficient to produce symptoms, and that it does not increase in the plant in the absence of the virus.

Further evidence that the leaf-deforming principle is not the tomatomosaic virus was shown by the fact that the inoculation of healthy tomato plants with the leaf-deforming principle combined with the potato virus did not produce the disease known as streak, whereas streak would have been produced had the tomato-mosaic virus been present. Likewise, inoculation with the juice of plants showing only the leaf deformities and with the potato virus failed to produce streak.

The leaf-deforming principle was not destroyed by heating for $2\frac{1}{2}$ hours at 126° C. This indicates that it is not of the nature of a virus and that it is nonliving.

In general, the most active filtrates were prepared by permitting the juice from mosaic plants to autolyze or ferment. This suggests the possibility that the leaf-deforming principle may be formed through decomposition of the constituents of the plant juice and may possibly have no relation to the presence of the virus. However, filtrates similarly prepared from the juice of healthy tomato plants, when inoculated into young, growing tomato plants, failed to produce any leaf-deformity symptoms.

These results indicate that the leaf-deforming principle is produced only in the diseased tomato tissues. Whether the principle is a product of the virus or merely a decomposition product of the constituents of the mosaic plant is uncertain.

A HITHERTO UNREPORTED DISEASE OF MAIZE AND BEANS

W. W. MACKIE

In September, 1929, the writer found what appeared to be a hitherto unreported disease in maize in the experimental plots on the University Farm at Davis. Soon after, the same disease symptoms were noted on common beans, *Phaseolus vulgaris*, in the plots and also on Blackeyes, *Vigna sinensis*, at Hilmar, in the San Joaquin Valley. Specimens sent to Helen Johann, pathologist in the Division of Cereal Crops and Diseases of the United States Department of Agriculture, were identified as *Sclerotium bataticola* Taubenhaus. J. J. Taubenhaus, pathologist of the Texas experiment station, who first discovered and named this fungus in New Jersey in 1913 (15), confirmed the identification.

Appearance of the disease on maize. Charcoal rot, as it was first named by Taubenhaus, is not manifest in the maize plant until the latter approaches maturity. At this time the affected plant shows signs of premature ripening and later may bend or break just above the crown, causing severe lodging. When the stalk is split the pith and fibers appear as though dusted with fine particles of charcoal (Fig. 1, D). These black bodies are small sclerotia, varying from 1/20 to 1/5 mm. in diameter. This condition extends into the roots for an undetermined distance, but at least 12 in. or The black sclerotia may be found in the stalk to a height of 8 or 10 in. but have not been found beyond this point. The nodes of the infected area are darkened and frequently show a dark pink discoloration (Fig. This color does not always occur and in most cases can be ascribed to the effects of Fusarium moniliforme, which is very common in California The association of the 2 fungi in the maize plant is common, but apparently association is not necessary for both are found existing one without the other (Fig. 1, A and C).

Within the cornstalk the black sclerotia are found more densely clustered about the vascular bundles to which they appear attached (Fig. 1, E). In the inner medulla or pith, sclerotia appear in fewer numbers. Sclerotia are not found attached to or included in the primary cortex (Fig. 1, D).

The action of the fungus upon the plant is seen in the interference with the passage of water in the xylem, but soon the elaborated materials in the phloem are affected. This combination of injury causes wilting, premature ripening, and shriveled or underdeveloped kernels. At this time the pith disintegrates and the vascular bundles separate into single strands (Fig. 1, A and C). This condition soon leads to the lodging of the plant.

Charcoal rot in beans. The appearance of charcoal rot in bean fields is first seen when the plants are nearly full-grown. At this time a tendincy

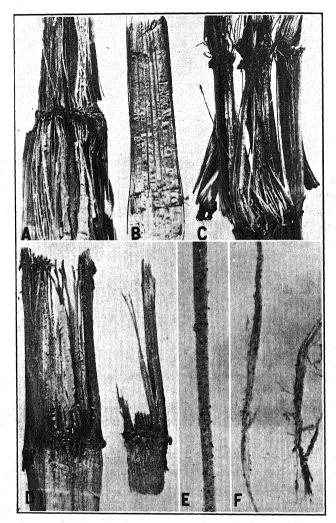


Fig. 1. A-D. Maize stalks. Disintegration of pith and separation of bundles due to Rhizoctonia bataticola alone in A and to Fusarium moniliforme alone in C. The stalks shown in B are free from disease. Both fungi are present in D. The sclerotia in the internodes belong to Rhizoctonia, but most of the discoloring, which is pink, in the nodes is due to Fusarium. E. Vascular bundle of maize with attached sclerotia. F. Vascular bundles of beans with attached sclerotia.

to wilting, yellowing of the natural, healthy green color, and premature ripening is noticed. Many fields presented the characteristic oil spots that are associated with the spread of a disease from a central point of infestation.

As in the case of maize, charcoal rot in the bean plant is found commonly associated with Fusarium. The Fusarium is readily seen in the pink discolorations, which may or may not occur in plants affected by the charcoal rot alone. Inasmuch as the Fusarium wilt attacks early in the life of the bean host and the charcoal rot appears late, the latter has been classed by some pathologists as a saprophyte (6). The fact remains, however, that the most deadly injury from the charcoal rot appears when the Fusarium wilt is not present.

Upon dissecting the bean plant, the diseased area, as indicated by the presence of the black sclerotia, extends to 8 or 10 in. above the soil, though in Blackeyes a greater height is attained. The roots are affected to an undetermined distance.

The stele is usually found crowded with the black sclerotia, which are attached to the vascular bundles as in maize (Fig. 1, E). In beans, as in maize, the water-carrying vessels appear to suffer the first attack, followed by the injury to the phloem. The wilting in beans is more pronounced than in maize and may induce the farmer to believe that Fusarium is the cause. Premature ripening is very distinct and is seen in the yellowing of the normal green in the leaves, a stiffening or hardening of the stelar structure. Apparently the charcoal-rot mycelium first attacks the water-carrying vessels, for in the stele or woody portion sclerotia are always found, usually in vast numbers, markedly darkening it (Fig. 2). The cambium and meristematic layers are then infected, and here dense clusters of sclerotia may be



Fig. 2. A. Sclerotia on the bast fibers of the common bean, *Phaseolus vulgaris*. B. Black sclerotia beneath the epidermis of the Blackeye cowpea, *Vigna sinensis*.

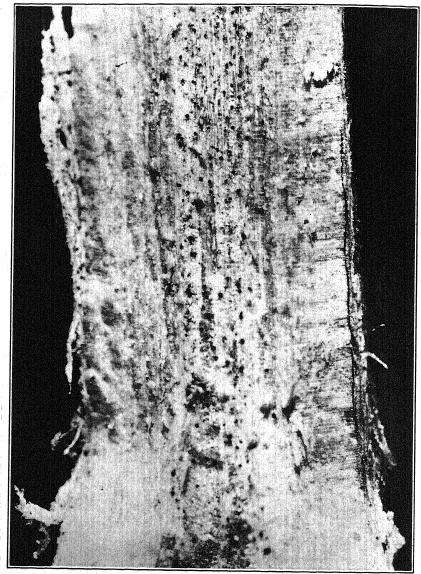


Fig. 3. Bean stem, showing the spread of charcoal rot in the hollow of the stem.

found in the more severely attacked plants (Fig. 2, A). This same condition holds for both roots and stems (Fig. 4). The medulla or pithy portion contains fewer sclerotia than the woody fibers of the state. The sclerotia are attached to the woody fiber of the inner structure and to the bast fibers of the outerbark. The epidermis is not directly attacked, but

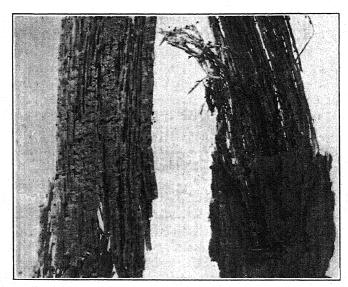


Fig. 4. Bean root, *Phaseolus vulgaris*, showing concentration of sclerotia in the fibers just beneath the epidermis.

in extreme cases (Fig. 2, B) the black sclerotia may be seen through the epidermis. The result of this attack is that the diseased plant does not reach its full stature and the seed is reduced in size, corresponding to the completeness of the attack.

Method of infection and spread. Charcoal rot on maize and beans is carried by the sclerotia alone, so far as observed in California. The sclerotia culture readily on several kinds of agar media and within a week abundant sclerotia are formed. There is a general agreement among authorities—Briton-Jones (4), Butler (5), Small (13), and Reichert and Hellinger (10)—that the charcoal-rot-fungus mycelium enters the small feeding roots and travels toward the crown. Martin (8) and Haigh (7) demonstrated that infection is readily induced in the upper portions of certain plants by artificial inoculations in wounds. The rate of progress in beans is considered rapid by Ashby (1), Reichert and Hellinger (10), and others, but in woody perennial plants it may be very slow, according to Small (14), Butler (5), and other East-Indian authorities (11).

The oil-spot spread of the disease observed in bean fields of California indicates that the mycelium may spread through the soil from plant to plant. Laboratory cultures to secure artificial infection are usually not satisfactory. Sclerotia appear to be the only fruiting bodies occurring on beans and corn in California. The disease, therefore, is carried from sclerotia to sclerotia.

PHYTOPATHOLOGY

The pyenial stage, Macrophomina phaseoli (Maubl.) Ashby, found by Ashby (1) and Haigh (7) to occur in beans and sunflowers in Asia, has not yet been found by the writer in California. The sclerotia may remain viable over one or more years, imbedded in the plant tissues or elsewhere. Because of their small size (1/20-1/5 mm.) they may be carried on the seed. It is doubtful if the fungus can be carried internally in the bean seed in the mycelial stage. As the organism apparently does not attack except in the warm summer period, the sclerotia apparently do not germinate except at this time and, hence, overwinter readily.

Hosts. Since 1913, when Taubenhaus (15) discovered charcoal rot in sweet potatoes in New Jersey, the fungus has been found on more than 50 different species hosts wide-spread in the East Indies, West Indies, Rhodesia, Uganda, Egypt, Palestine, India, Formosa, and adjacent Asiatic countries (2, 3, 4, 9, 12, 14). Monocotyledonous and dicotyledonous plants are attacked, annuals and perennials, soft and woody plants in subtropical and tropical areas, principally. The hosts representing crops of interest to the United States include cotton, tobacco, beans, peppers, limes, lemons, oranges, tomatoes, sweet potatoes, cypress, sunflowers, strawberries, roses, and maize. That a single form attacks all these hosts appears unlikely. Haigh (7) has found at least three classes, as indicated by the consistent differences in the size of the sclerotia that occur over several generations.

TAXONOMY

Ashby (1, p. 145) gives synonyms as follows:

Macrophoma Phaseoli Maubl. (1905).
Sclerotium bataticola Taub. (1913).
Macrophoma Corchori Saw. (1916).
Macrophoma Cajani Syd. and Butl. (1916).
Macrophomina philippinensis Petr. (1923).
Rhizoctonia lamellifera Small (1924).
Rhizoctonia bataticola (Taub.) Butl. (1925).
Dothiorella Cajani Syd. and Butl. (1925).
Macrophoma Sesami Saw. (1922).

The form bearing only sclerotia is generally accepted to be *Rhizoctonia bataticola* (Taubenhaus) Butler. The pycnidial form has been identified by Haigh (7) as *Macrophomina Phaseoli* Maublanc. The typical sclerotial form of *R. bataticola* was recovered by Haigh from 50 hosts, but the pycnidial form, from beans and sunflowers only. The pycnidia always give rise to the sclerotia, but the sclerotia do not commonly give rise to pycnidia. The writer has found the sclerotial form only in California.

In maize all subspecies appear susceptible. In the Bay Region, Yolo,

and San Joaquin counties, charcoal rot has been found abundant in maize, but the extent of the area affected is yet unknown.

Charcoal rot has been found in beans from Imperial County in the extreme south of California to Shasta County in the north and in the coastal as well as interior regions. All species of beans grown commercially in California, including Phaseolus vulgaris, P. multiflorus, P. lunatus sieva, and Vigna sinensis, but excepting P. lunatus, were found attacked. Among the common beans, P. vulgaris, many varieties, selections, and hybrids appear highly resistant. The Blackeye cowpea, V. sinensis, is highly susceptible, but other cowpeas have been found free from attack. The Large Lima, P. lunatus, has not been found affected, but one field of Baby Limas, P. lunatus sieva, yielded typical diseased plants. The Hopi Limas, which belong to this species, were not observed attacked. All of the few varieties of Butter bean, P. multiflorus, under observation were found very susceptible.

SUMMARY AND CONCLUSIONS

- 1. A fungus disease new to California has been found on maize and beans and the causative organism has been identified as *Rhizoctonia bataticola* (Taubenhaus) Butler.
 - 2. The same fungus has been found on more than 50 plant species.
- 3. Charcoal rot, the name given the disease caused by the sclerotial form by Taubenhaus, is common in the East Indies and in the subtropical and tropical regions of Asia. It has been found in Egypt, Uganda, and Rhodesia in Africa, the West Indies, and the United States.
- 4. The pycnidial form is *Macrophomina phaseoli* Maublanc. The pycnidial stage always gives rise to the sclerotial but the sclerotial stage in many cases does not give rise to the pycnidial.
- 5. Usually, charcoal rot is carried from sclerotia to sclerotia without other spore forms, making it readily separable from most other associated fungi.
- 6. The fungus attack is made through the young feeding roots in the soil, where it is carried over by the sclerotia. Entrance to older tissue may follow wounds.
- 7. The only known means for combating this disease in maize and beans lies in breeding for varietal resistance. This is possible as highly resistant varieties have been found.

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THE ADHERENCE OF COPPER DUSTS TO FOLIAGE

L. R. STREETER, E. O. MADER, AND F. J. KOKOSKI

INTRODUCTION

Dusting preparations containing copper sulphate monohydrate (CuSO₄: H₂O) and calcium hydroxide are frequently used as substitutes for Bordeaux mixtures. Many experiments have been reported in which such dusts are compared to Bordeaux mixture for effectiveness in controlling insects and diseases injurious to potato foliage. When copper sulphate monohydrate hydrated lime dust is used, it is assumed that a reaction takes place on the foliage by means of which Bordeaux mixture or some substance with comparable protective properties is formed.

Folsom and Bonde¹ showed by analysis of potato foliage for copper that the copper in Bordeaux mixture adheres better than that in copper-lime dust.

Boyd² has shown that, under both greenhouse and field conditions, dusts adhere much better when applied to thoroughly moist leaves than when applied to dry leaves and that dusts applied to moist foliage were more effective in controlling diseases and insect pests than when applied to dry foliage.

EXPERIMENTAL STUDIES

In connection with more recent experiments in the use of copper dusts by the Department of Plant Pathology at Cornell University several experiments have been carried on to determine the effect of moisture and time of application upon the adherence of copper to potato foliage. The dusts were applied with a traction duster to both moist and dry foliage and the conditions noted for each separate test. The preparations used contained 81 parts of hydrated lime and 19 parts of copper sulphate monohydrate. For each treatment 200 gm. of leaves were collected and analyzed for adhering copper. The amount of copper found is expressed in mgm. per 100 gm. of leaves. In each test 2 dust mixtures were used and in this discussion they will be designated as Dusts 1 and 2, respectively.

In the first experiment, July 15, applications were made at 4:30 a.m. and 8:00 a.m. On this date dew appeared at 3:30 a.m. and disappeared at 7:00 a.m. Rain on July 24 amounted to 0.98 in. The results of analyses for copper are given in table 1.

¹ Folsom, Donald, and Reiner Bonde. Potato spraying and dusting experiments 1921 to 1925. Maine Agr. Exp. Sta. Bul. 334. 1926.

² Boyd, O. C. The relative efficiency of some copper dusts and sprays in the control of potato diseases and insect pests. N. Y. (Cornell) Agr. Exp. Sta. Bul. 451. 1926.

TABLE 1.—The amount of copper present on leaves of potato dusted with 81 parts hydrated lime and 19 parts copper sulphate monohydrate expressed in mgm. per 100 gm. of leaves

		Date o	of collection of s	ample
Hour of application	Dust	7/15	7/21	7/24
pacouson		Milligrams of	copper per 100	grams leaves
4:30 a.m.	Dust No. 1	35 30	7 5	trace
8:00 a.m.	""1	22	trace	
	2	17	•	

In experiment No. 2 dust applications were made on Aug. 3. At the time of application no dew was present, but a mist-like rain occurred immediately after the dust was applied and the foliage became thoroughly wetted. The rain ceased after 30 min. and, after the foliage became dry, samples of leaves were taken for analysis. Rain on Aug. 3 amounted to 0.40 in. and on Aug. 11 to 0.42 in. The results of sampling on these separate dates are given in table 2.

TABLE 2.—The amount of copper present on leaves of potato dusted with 81 parts hydrated lime and 19 parts copper sulphate monohydrate expressed in mgm. per 100 gm. of leaves

	Date of collection of sample				
Hour of application Dust	8/3 8/8 8/12				
	Milligrams of copper per 100 grams leaves				
8:00 a.m. Dust No. 1	35 30 7.5				
	30 22 2.5				

In experiment No. 3 applications were made at 12:00 p. m., Aug. 12 and 2:00 a. m., 4:00 a. m., and 6:00 a. m., Aug. 13. Dew appeared at 3:00 a. m. and disappeared at 8:00 a. m. Rain on Aug. 16 amounted to 0.12 in. and on Aug. 22 to 0.54 in. The results of samplings on the 4 different days are given in table 3.

In experiment No. 4 dusts were applied at 8:00 p. m. and 12:00 m. on Aug. 21 and 4:00 a. m. and 8:00 a. m. on Aug. 22. Dew appeared at 4:00 a. m. and disappeared at 11:00 a. m. Rain on Aug. 22 amounted to 0.54 in. and on Aug. 27 to 0.14 in. Results of analyses of samples taken on 3 different days are given in table 4.

In experiment No. 5 dust applications were made at 12:00 m. Aug. 29 and 4:00 a. m. and 8:00 a. m. Aug. 30. Dew appeared at 4:00 a. m.

TABLE 3.—The amount of copper present on leaves of potato dusted with 81 parts hydrated lime and 19 parts copper sulphate monohydrate expressed in mgm. per 100 gm. of leaves

		Date of collection of sample			
Hour of application	Dust	8/13	8/17	8/19	8/22
		Milligrams of copper per 100 grams leaves			
12:00 p.m.	Dust No. 1	34	12.5	trace	0
	" " 2	36	10		0
2:00 a.m.	" " 1	39	15	2.5	0
	" " 2	40	10	trace	0
4:00 a.m.	" " 1	45	25	10	trace
	" " 2	41	22	4	0
6:00 a.m.		45	27.5	15	2
	" " 2	43	22	8	trace

TABLE 4.—The amount of copper present on leaves of potato dusted with 81 parts hydrated lime and 19 parts copper sulphate monohydrate expressed in mgm. per 100 gm. of leaves

		Date of collection of sample			
Hour of application	Dust	8/22 8/25 8/28			
		Milligrams of copper per 100 grams leaves			
8: 00 p.m.	Dust No. 1	12 0 0			
	((((2	16 trace 0			
12:00 p.m.	" " 1	21 " 0			
		23 "			
4:00 a.m.	" " 1	40 10 trace			
	" " 2	35 15 "			
8:00 a.m.	" 1	47 35 20			
	2	40 12.5			

and disappeared at 10:30 a.m. No rain occurred during the period of this experiment. The results of analyses of samples taken on these days are given in table 5.

These experiments indicate that the presence of moisture on the foliage is essential to good adhesion of the copper-lime dust, as the adherence of copper is closely correlated with the presence or absence of dew. The writers are of the opinion that the difference in adherence is explained by the fact that the calcium hydroxide is changed to carbonate when moisture is absent, with the result that a good adhering film is not formed.

To test the theory that poor adhesion is due to the conversion of the calcium hydroxide to calcium carbonate, a few laboratory experiments have been performed. A dust was prepared by mixing 20 parts of copper sul-

TABLE 5.—The amount of copper present on leaves of potato dusted with 81 parts hydrated lime and 19 parts copper sulphate monohydrate expressed in mgm. per 100 gm. of leaves

		Date of collection of sample			
Hour of application	Dust	8/30	9/3	9/7	
pireation		Milligrams of copper per 100 grams leaves			
12:00 m.	Dust No. 1	25	10	trace	
		27	9	0	
4:00 a.m.	" " 1	45	22	7	
	" " 2	40	20	5	
8:00 a.m.	" " 1	55	49	15	
		52	37.5	12.5	

phate monohydrate with 80 parts of calcium hydrate. A part of this mixture was exposed for 5 hrs. to air at 70° F. and 80 per cent relative humidity. After exposure this fraction and a part of the original mixture were dusted onto glass plates previously moistened with atomized water. After drying, artificial rain was applied and the adhering copper determined. The results of this experiment are given in table 6. These results

TABLE 6.—The amount of copper present on glass plates previously moistened with water and then dusted with 20 parts copper sulphate monohydrate and 80 parts calcium hydrate, and subsequently exposed to artificial rain

Experiment	Original dust	After 5-hr. exposure to air		
No.	Gm.	Gm.		
1	0.040	0.022		
2	0.033	0.023		
3	0.035	0.019		
4	0.014	0.005		

show further that a certain amount of water of condensation is essential to good adherence at the time of application of the dust.

The above experiments indicate that the change from calcium hydroxide to calcium carbonate may take place in a few hours, but in order to obtain some definite information on the rate of conversion under uniform conditions of temperature and humidity a series of experiments have been carried out in the laboratory.

For these tests high-grade commercial hydrated lime, 99 per cent of which in each case passed through a 325-mesh sieve were used. The hydrated lime powders were dusted onto paper-covered trays and placed in an air-conditioning cabinet. The dust layers were approximately \(\frac{1}{3} \) mm. in

thickness. A moderate air circulation was maintained and also a constant change of air from outside the cabinet. The rate of conversion under the several conditions maintained are given graphically in figure 1.

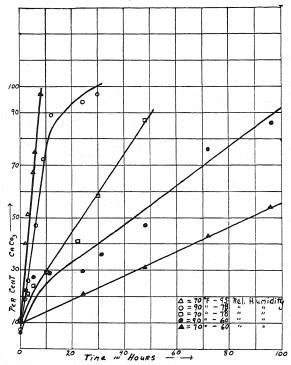


Fig. 1. The effect of temperature and humidity on the rate of carbonation of calcium hydrate.

The conditions of the experiment are not identical with those encountered in the field, but the data give some basis for estimating time during which lime may be expected to function as calcium hydroxide.

These data clearly show that both temperature and humidity are important factors in the conversion of calcium hydroxide to calcium carbonate. It can be concluded further that conditions favoring the conversion of thin layers of hydrated lime to calcium carbonate within a few hours are common in midsummer. These data further emphasize the importance of moisture being present at the time of or soon after the application of dust mixtures, such as copper-lime dusts, from which Bordeaux mixture is supposed to form on the foliage. It is evident that when reactions involving lime take place on foliage, the calcium carbonate as well as the hydroxide content of the lime must be considered.

SUMMARY

The data presented in the foregoing show the importance of timing applications of copper-lime dusts to coincide as closely as possible with the occurrence of water of condensation on the foliage.

It is evident that a high relative humidity increases the rate of carbonation of the hydroxide but does not supply sufficient moisture to convert the dust mixture into a good, adhering film or membrane.

The conversion of calcium hydroxide to calcium carbonate may take place in a few hours or it may take several days, depending on the temperature and relative humidity of the air.

GENEVA AND ITHACA. NEW YORK.

THE EFFECT OF DELAYED PLANTING ON THE CONTROL OF BUNT BY COPPER CARBONATE DUST¹

H. H. FLOR

INTRODUCTION

Many of the popular publications advocating the copper carbonate dust method of seed treatment for control of wheat bunt state, as one of the advantages of this treatment, that it can be done during the slack season and the seed stored for later planting. Heald and Smith² showed that treated seed can be stored without injury to germination. However, the writer is unaware of the publication of the results of any test to determine the effect of the lapse of time between seed treatment and planting on the effectiveness of the copper carbonate treatment.

MATERIALS AND METHODS

The effect of planting at weekly intervals following seed treatment was studied at Pullman, Washington, during the season of 1930–31. Seed of Hybrid 128 (C. I. 4512) was inoculated with fresh bunt spores in the proportion of 1 gm. of spores to 100 gm. of wheat. The inoculum was obtained from E. F. Gaines and was composed of equal parts by weight of 3 forms of Tilletia tritici (Bjerk.) Wint. and 2 forms of T. levis Kühn. Hybrid 128 is susceptible to all of these forms. The inoculated seed was divided into 3 lots and on September 8, 1930, 1 portion was treated with copper carbonate dust, of 50–52 per cent copper content, at the rate of 4 oz. per bu.; the 2nd lot was soaked for 1 hr. in a 1:320 solution of commercial formaldehyde, covered over night, and then dried. The 3rd lot was not treated. On the following day the seed was put up in packets for rod-row planting. All packets were stored in the laboratory at room temperature and taken to the field as needed for planting.

The seed was planted in the agronomy nursery in plots of 3 rod rows. Four plots of each treatment were planted on each date of seeding. At maturity the bunted and bunt-free heads in the center row of each plot were counted. Soil-thermograph records were taken of soil temperature at the depth of planting, which was 2 to 3 in. below the surface. The 1st sowing was made on September 12, 1930, just after the fall rains had started and while the soil was wet and sticky. The 2nd sowing was made

¹ Cooperative investigations between the Washington Agricultural Experiment Station and the Division of Cereal Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture.

² Heald, F. D., and L. J. Smith. The dusting of wheat for bunt or stinking smut. Wash. Agr. Exp. Sta. Bul. 171. 1922.

on September 15, after the soil had dried out for 3 days. Sowings were made every 7th day thereafter until prevented by snow and frozen ground.

RESULTS

The results of this test are given in table 1.

The small amount of bunt in the inoculated check plots of the 1st and last sowings indicates that earlier or later sowings would probably have been of little value, as conditions were unfavorable for bunt infection. Favorable conditions for bunt infection existed during the period from the 3rd sowing on September 22 until the 9th, on November 3, as shown by the high percentage of bunted heads produced in the inoculated plots.

The most interesting feature of this test was the gradual increase of bunt in the plots sown with copper carbonate-treated seed during the period covered by the first 9 sowings, while the formaldehyde treatment was uniformly effective throughout the test. This is brought out more clearly by a comparison of the 2 columns showing the proportional reduction of bunt by the 2 treatments. During the period covered by the first 5 sowings, the copper carbonate and the formaldehyde treatments were about equally effective. The formaldehyde treatment usually gave better control, but the differences were not significant. The gradual increase in percentage of bunt in both treatments during this period may be accounted for by the increasing severity of soil infestation. Heald and Gaines² have shown that soil infestation due to wind-blown spores, at Pullman is usually on the increase during this period and reaches its maximum during the 1st week in October.

All sowings subsequent to that of October 6 show a great difference in the effectiveness of the 2 treatments. The formaldehyde treatment varied in efficiency from 92.3 to 100 per cent, when compared with the percentages of bunt from nontreated seed, while the efficiency of the copper carbonate treatment varied from 81.0 to 64.8 per cent. The differences in the effectiveness of the 2 treatments in the last 5 sowings were many times the probable error in every instance and, therefore, were undoubtedly significant. The temperature at the time of the 10th sowing was too cold for good bunt infection, as the plots sown with untreated seed had but 21.3 per cent infected heads as compared with 89.3 per cent in the sowing of the previous week. The copper carbonate treatment in the last sowing reduced bunt to 5.7 per cent, or 25.8 per cent of that in the inoculated non-treated check. This was the only sowing in which the copper carbonate treatment did not show a decrease in effectiveness when compared with the one made the previous week, but, in view of the decrease in bunt in the check

³ Heald, F. D., and E. F. Gaines. The control of bunt or stinking smut of wheat. Wash. Agr. Exp. Sta. Bul. 241. 1930.

TABLE 1,-The influence of the date of planting on the effectiveness of copper carbonate and formaldehyde seed treatments for the control of wheat bunt

Seed of Hybrid 128 wheat, inoculated with Tilletia levis and T. tritics and treated September	8, 1930, with 50-52 per cent copper carbonate, 4 oz. per bu., and 1: 320 formaldehyde solution	by soaking seed 1 hr. and covering overnight]

		Soil	Soil temperature ^b	reb	Average p	Average percentage of bunted heads from inoculated seed	unted heads seed	Proportionate control by	e control by
Seed sown	Precipita-		•			Treate	Treated with—		
		Мах.	Min.	Ave.	Untreated	Copper carbonate	Formalde- hyde	Copper earbonate	Formalde- hyde
		°F.	oF.	oF.					
Sept. 12			1		14.6 ± 3.89	0.8 ± 0.24	0.6 ± 0.14	94.5	95.9
15	0.25	82	13	99	45.8 ± 2.88	2.7 ± 0.17	3.4 ± 0.68	94.2	92.6
22	0.03	79	46	09	87.3 ± 3.00	6.9 ± 0.93	1.9 ± 0.24	92.1	8.76
29	0.32	2.2	46	69	84.3 ± 2.19	10.2 ± 2.20	9.7 ± 0.90	87.3	88.5
Oct. 6	0.23	•		ı	93.3 ± 0.88	13.2 ± 1.02	10.0 ± 1.48	85.8	80.3
13	0.32	09	37	ु इ	92.9 ± 0.69	17.6 ± 0.96	5.1 ± 1.20	81.0	94.5
20	0.40	57	36	124	93.5 ± 1.28	22.3 ± 1.78	4.7 ± 1.23	76.1	95.0
27	0.29	09	35	46	93.6 ± 0.54	28.3 ± 1.90	6.3 ± 0.84	69.7	93.3
Nov. 3	00.0	55	36	46	89.3 ± 1.34	31.4 ± 0.53	6.9 ± 0.74	64.8	92.3
10	0.14	20	58	35	21.3 ± 1.53	5.7 ± 0.90	0.0 ± 0.0	74.2	100.0

a For week ending on dates indicated.

b Average for first weeks following seeding, taken at depth of planting.

e No data as thermograph failed to record the entire week.

and the perfect control given by the formaldehyde treatment, it is probable that this difference was due to unfavorable environmental conditions for bunt infection rather than to an increase in the toxic action of the copper carbonate.

It is difficult to account for the gradual decrease in efficiency of the copper carbonate dust treatment with each successive sowing over a period of approximately 2 months. There are a number of possible explanations: soil moisture; soil temperature; and a gradual decrease in the toxicity of the copper carbonate when applied to the wheat seed and under the conditions of storage. Unfortunately, soil-moisture records were not taken. but the rainfall records of the experiment station were available. A study of the rainfall records shows that it is doubtful if soil moisture was an important factor. Rains were rather generally distributed over the planting period except for the 9th sowing. No rain had fallen for 9 days prior to or for 6 days subsequent to this sowing, yet there was no difference in the trend of bunt infection. The plots sown with nontreated and formaldehydetreated seed had approximately the same percentage of bunt as those of the previous sowing, while the plot sown with copper carbonate-treated seed had a small increase in the percentage of bunt. It is possible that soil temperature may have been an important factor in the gradual decrease in the effectiveness of the copper carbonate treatment, for there was a decline from an average of 66° F. during the first week of the periodic sowings to 35° F. during the last week. However, in view of the amount of work that has been done with the copper carbonate-dust treatment by numerous investigators throughout the world, who have found the treatment effective under a wide range of conditions, it is difficult to believe that the production of 31.4 per cent bunted heads in the 9th sowing could be accounted for by the lowered temperature. Further evidence that low soil temperature was not the principal factor in the gradual decreased effectiveness of the copper carbonate treatment is shown by an examination of the soil-temperature records. The maximum, minimum, and average soil temperatures for the weekly periods following the 6th, 7th, 8th, and 9th sowings were essentially similar; yet the percentage of bunted heads in the copper carbonate-treated plots gradually increased from 17.6 per cent in the 6th to 31.4 per cent in the 9th sowing. During this period differences in percentage of bunt in the sowings of inoculated nontreated seed and formaldehyde-treated seed were not significant. Consequently, the most plausible explanation for the relative ineffectiveness of the copper carbonate treatment in the later sowings appears to be the occurrence of something that caused a decrease in the toxicity of the chemical. decreased toxicity was progressive, becoming more pronounced with the increased interval between date of treatment and that of sowing. The cause of this change in the toxicity of the copper carbonate, whether physical, chemical or biological has not been studied.

Although the seed used in these tests was smutted much more heavily than seed that would ordinarily be used in commercial plantings and although the test was limited to a 1-year trial, it is thought that the results may explain, to some extent, the numerous failures that have been reported in the commercial use of the copper carbonate-dust treatment. Undoubtedly, many of these cases of failure were caused by inefficient methods of seed treatment, which failed to distribute the dust evenly over the seed. However, the results of this test indicate that some of the failures may have been due to the practice of treating the seed in the slack season and storing until time of sowing, as advocated in many of the popular articles on this method of seed treatment. There is need of a more thorough study of the effect that the interval of time elapsing between seed treatment and sowing has on the effectiveness of the copper carbonate method of treatment for control of bunt.

SUMMARY

The comparative efficiency of the formaldehyde-soak and the copper carbonate-dust treatments was tested by periodic sowings at weekly intervals. The effectiveness of the formaldehyde treatment was fairly constant, varying from 88.5 per cent in the 4th planting to 100 per cent in the 10th. The effectiveness of the copper carbonate treatment decreased with each successive sowing from 94.5 per cent in the 1st to 64.8 per cent in the 9th. Up to and including the 5th weekly sowing, the differences in effectiveness of the 2 treatments were not significant, but in sowings made later the copper carbonate was decidedly inferior.

⁴ Haskell, R. J., R. C. Rose, W. E. Brentzel, E. A. Walker, and Waldo Kidder. Why so much smut in spring wheat? U. S. Dept. Agr., Bur. Plant Indus. Plant Dis. Rptr. Sup. 77: 97–139. 1930. (Mimeographed).



THE BEHAVIOR OF BACILLUS AMYLOVORUS IN SOIL

P. A. ARK¹

Comparatively few experiments seem to have been made bearing on the relation of *Bacillus amylovorus* (Burr.) Tree. to the soil. Arthur² reported in 1886 that the organism grew "with moderate readiness" in soil extract. Waite,³ in 1895, presented the following summary statement: "It has been claimed that the blight microbe lives over winter in the soil and for a long time the writer supposed this to be the case, but after careful investigation the idea was abandoned for in no instance could it be found there."

The present paper deals with a somewhat specialized technique for the separation of the organism from the soil and the application of this technique to some soils infested by both artificial and natural means.

MATERIALS AND METHODS

The technique employed here diverged from that in general use in bacteriological laboratories in two particulars and is partly an application of Hotson's4 method for isolating the pear-blight organism. First, in order to inhibit the gram-positive organisms present in large numbers in unsterilized soil, Patel's oxgall medium⁵ was used with the following modification: Crystal violet 1: 100,000 was used instead of 1: 500,000. Second, surface-sterilized green pear fruits or portions of these were punctured with needles and immersed in suspensions of the soils to be tested and then removed and incubated at a temperature favorable for Bacillus amylovorus. This not only serves to increase the blight organism to the point where it can be readily isolated by ordinary culture methods but also results directly in symptoms of blight accompanied by the characteristic bacterial exudate. Of 35 cultures of bacteria tested on pear fruits, including 15 species, namely, Bacterium juglandis (Pierce) E. F. Sm., Bact. viridilividum Brown, Bact. savastanoi E. F. Sm., Bact. phaseoli E. F. Sm., Bact. medicaginis var. phaseolicola (Burkh.) Link & Hall, Aplanobacter michiganense (E. F. Sm.) E. F. Sm., Ps. cerasus Griffin, Bact. tumefaciens E. F. Sm. & Town., Bacillus

- ¹ This work was done under the direction of Professor R. E. Smith of the Division of Plant Pathology, University of California.
- ² Arthur, J. C. Pear blight. *In* Report of the botanist. N. Y. (Geneva) Agr. Exp. Sta. Ann. Rpt. 5 (1886): 259-273. 1887.
- ³ Waite, M. B. The cause and prevention of pear blight. U. S. Dept. Agr. Yearbook 1895: 295-300. 1896.
 - 4 Hotson, J. W. Fire blight on cherries. Phytopath. 5: 312-316. 1915.
- ⁵ Patel, M. K. An improved method of isolating *Pseudomonas tumefaciens* Sm. and Town. Phytopath. 16: 577. 1926.

carotovorus L. R. Jones, Bact. aroideae (Towns.) Stapp, B. subtilis (Ehrenb.) Cohn, Bact. coli (Esch.) Mig., B. megatherium de Bary, B. faecalis alkaligines Cast. & Chalm., Staphylococcus albus Rosenb., none produced an exudate that need be confused with that of the blight organism. (Fig. 1.) More than 750 uninoculated fruits with and without puncturing were incubated as controls in the course of these experiments, but none of them developed any symptom of blight.

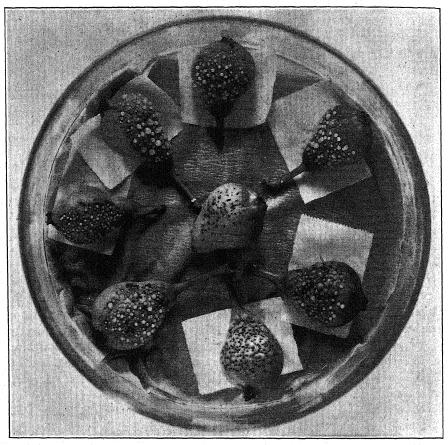


Fig. 1. Whole immature pears with profuse bacterial exudate due to immersion in water suspension of natural soil taken from beneath blighted tree. The pear in the center is the control.

For the preliminary laboratory tests, soils of 3 diverse types were infested: loam surface soil, clay subsoil and sand; the last containing very little organic matter. These were air-dried, passed through a 20-mesh screen, and placed in 150-gm. lots in 300 cc. flasks. Soils were infested by

moistening throughout with beef-peptone-broth cultures of the blight organism. Sterile water and implements were used in suspending and handling the soils. For each soil, 3 flasks were steam-sterilized before sowing with the blight organism and 3 were infested without sterilization. One flask of each lot was incubated at each of the 3 temperatures, 8°, 21°, and 28° C. Both the crystal violet-bile agar and the pear fruits were used in recovering the organism from artificially infested soil.

CULTURES FROM ARTIFICIALLY INFESTED SOIL

Cultures were made from the soils at intervals of 2 or 4 days during a period of 30 days and at long intervals afterward. For the sterilized soils the numbers of organisms per cubic centimeter of soil were computed for each sample. The numbers declined rapidly during the first 2 to 4 days and gradually thereafter. It will be seen in table 1 that the loam and clay were more favorable to the persistence of the organism than the sand.

TABLE 1.—Longevity (in days) of Bacillus amylovorus in soils at various

Centigrade temperatures

Type of		Sterilized			Unsterilized	
soil	8°	21°	28°	8°	21°	28°
Loam	54	30	30	30	30	30
Clay	54	30	30	38	38	14
Sandy	18	18	0	14	22	18

CULTURES FROM NATURALLY INFESTED SOIL

During the summer, autumn, and early winter of 1931, cultures were made at irregular intervals from soil samples collected beneath blighted pear trees and other susceptible plants. In a few cases samples were taken at some distance from any blight infection. The results are summarized in table 2. The organism was obtained from a considerable number of samples up to the time of the autumn rains.

In several instances the organism was obtained from samples midway between tree rows and in one instance, on November 18, 1931, from an open field from which pear trees had been removed in March or April, 1931.

The soils were first thoroughly wet by rains that fell on November 16, 1931.

The identity of all the organisms was verified by examination in pure culture and all except those of the first lot of samples were tested on shoots of growing potted plants (*Cotoneaster pannosa*) in the greenhouse. For the first lot the organisms were reintroduced into green pear fruits and resulted

in typical symptoms on these. The tests on growing shoots have been limited, and it is probable that others of the cultures indicated in column 3, table 2, will produce infection when tested more extensively.

No relation is apparent thus far between the persistence of the organism and the type of soil (sandy loam to silt loam) or the depth of sampling (down to 5 or 6 in.).

	<u> </u>			
Date	No. of samples	Infection on pear fruit	Positive on shoots	Locality
7-8-31 } 7-23-31 }	50	8	Not tested	Alameda County
11-10-31	8a	7	7	Sacramento Valley
11-18-31	6b	5	4	Napa County
11-18-31	20c	14	3	Sacramento Valley
11-18-31	244	21	7	Sonoma County
12-10-31	5	0	Not tested	Santa Clara County
12-18-31	22	0	Not tested	Sacramento Valley

TABLE 2.—Cultures from naturally infested soils

SUMMARY

Bacillus amylovorus persisted for 54 days in sterilized soil and for 38 days in unsterilized soil in the laboratory.

The organism was obtained from orchard soils under conditions indicating persistence for at least several weeks in dry or moderately moist soils.

a One sample was taken between 3 trees. Positive on fruit and shoot.

^b One sample was taken between road and trees, 20 ft. from the nearest pear tree. Positive on fruit and shoot.

^c Four samples were taken from the area where the blighted trees were pulled out in April, 1931. These samples were negative for *Bacillus amylovorus*.

d One sample was taken from the bottom of a pit 12 in. deep from which an infected tree had been dug about 3 weeks before. The stump was almost oozing. Positive on pear fruit and shoot of Cotoneaster pannosa. One sample from open field was positive for Bacillus amylovorus.

THE PRODUCTION OF BUNT CHLAMYDOSPORES IN THE VEGETATIVE TISSUE OF THE WHEAT PLANT¹

H. H. FLOR

Wheat is subject to attack by 3 smuts: flag smut, loose smut, and bunt or stinking smut. Flag smut, caused by *Urocystis tritici*, Koern. is usually confined to the vegetative tissues, the spores being produced in slightly raised, white or dull gray stripes in the leaves and occasionally in the stem and inflorescence. Loose smut, caused by *Ustilago tritici* (Pers.), Jens., occurs normally in the inflorescence, converting all parts of it, both vegetative and reproductive, into the spore masses and leaving nothing but the bare rachis. Under greenhouse conditions and occasionally in the field, this smut may produce streaks of spores in the leaves. Bunt, or stinking smut caused by *Tilletia levis* Kühn and *T. tritici* (Bjerk.) Wint., usually has been considered to produce spores only in the ovaries of the host plant, thus differing from flag and loose smuts in that it did not produce spores in the vegetative tissues.

During the winter of 1930–1931 the writer inoculated seedlings of Prelude wheat with paired monosporidial cultures of each of the two bunt organisms. These plants were grown in a greenhouse at Arlington Farm, Virginia, maintained at 20° C., and subjected to a long day with artificial illumination. After the plants had headed, the soil was kept rather dry in order to hasten the ripening of infected heads. When the heads were nearly ripe the soil was given a thorough soaking so as to stimulate secondary stooling and see if infected shoots would not be produced by plants whose first heads had escaped infection, because field observations of partially smutted plants had shown the culms first produced were those that usually remained bunt-free. After the soaking, the plants revived and sent out new shoots from the basal nodes. A number of these shoots were dwarfed and deformed, and the leaves appeared to have wart-like smut galls on them (Fig. 1).

Microscopic examination showed that galls filled with chlamydospores of the bunt organisms had been produced in the parenchymatous tissues of both the leaves and stem. Spores produced in plants inoculated with cultures of *Tilletia tritici* had reticulately marked walls, while the walls of spores produced in plants inoculated with cultures of *T. levis* or the interspecific cross were smooth, like those produced in heads in the normal

¹ Cooperative investigations between the Washington Agricultural Experiment Station and the Division of Cereal Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture.

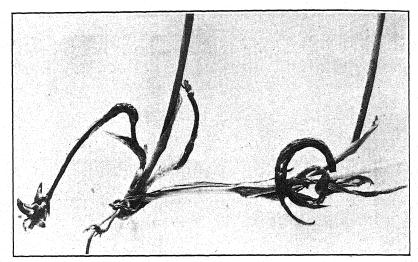


Fig. 1. Base of bunt-infected Prelude wheat plant, showing the secondary culms dwarfed and distorted due to the formation of bunt galls in the vegetative tissues.

The leaves on the culm at the extreme left are galled. ×2.

manner.² Spores produced in the vegetative tissue were slightly smaller than those in the head of the same culm, being $19.3\mu\pm0.097$, and $19.9\mu\pm0.086$, respectively.

A histological study was made of the galls in the leaf and stem tissues. The galls in the leaves occurred in irregular, noncontinuous strands varying from a few millimeters to 1 or 2 cm. in length. They extended from the leaf blade into the undifferentiated leaf and stem tissue at the node. Cross sections showed as many as 4 strands per leaf (Fig. 2, A, C). The strands of bunt spores varied greatly in thickness and diameter, attaining their maximum dimension in the leaf sheath (Fig. 2, B). They always occurred in the parenchymatous areas between or beneath the vascular bundles. Occasionally, fusion had taken place between strands, in which case the vascular bundles had been forced outward. The formation of the gall in the leaf and leaf sheath appeared to be accompanied by a slight hypertrophy (Fig. 2, B, C), but most of the deformity was due to the physical action of the expanding smut gall.

The galls in the stems were inconspicuous and probably would not have been noticed had not the stems been twisted and distorted. In the stems the galls occurred as a single strand made up of a series of swollen pockets connected by narrow necks. As this strand lay in one plane, it caused

² Flor, H. H. Heterothallism and hybridization in *Tilletia tritici* and *T. levis*. Jour. Agr. Res. 44: 49-58. 1932.

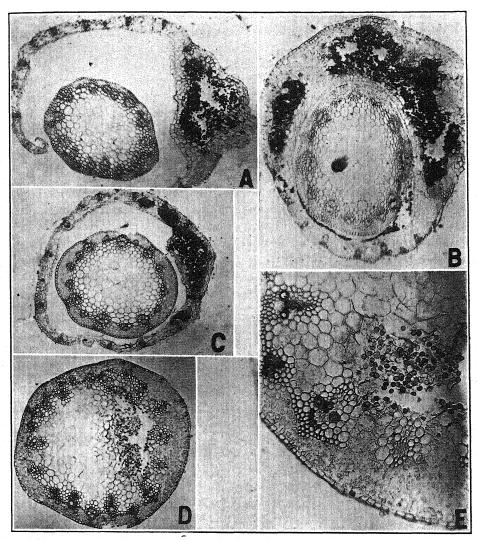


FIG. 2. A. Cross section of a bunt gall in the leaf blade, showing enlargement due to hypertrophy as well as to the mechanical expansion of the gall. ×25. B. Cross section of an infected leaf sheath. The bunt galls extend more than halfway around the sheath, which is enlarged in the galled regions due to the mechanical action of the expanding gall and to the excessive production of parenchymatous tissue. ×25. C. Cross section of a leaf just above the sheath, showing 4 strands of bunt spores in the tissue. ×25. D. Cross section of a galled stem, showing the bunt gall in the pith just beneath the vascular bundles. The cortical parenchyma adjacent to the gall has enlarged and the thick-wall cells that normally connect the vascular bundles and separate the pith from the cortical regions have disappeared. ×25. E. An enlargement of D, showing the inhibitory effect of the bunt gall on the development of thick walls in the conducting and supporting tissues adjacent to the gall and the disorganization of the cortical parenchyma. ×105.

the stem to twist greatly and in one instance to make a complete circle. Gall formation in the stem was accompanied by a small amount of hypertrophy, as shown by a thickening in the parenchymatous region lying adjacent to the gall and outside of the vascular bundles (Fig. 2, D). However, most of the deformity was due to the mechanical injury caused by the expansion of the gall in the tissue, as was the case in the leaf. Another anatomical change was the disappearance in the vicinity of the gall (Fig. 2, D, E) of the thick-wall cells that connect the vascular bundles and separate the central parenchymatous pith cells from the cortical parenchyma.

It is possible that bunt-spore formation in the vegetative tissues of the wheat plant may occur under certain conditions in the field but has not been noticed because of the inconspicuous symptoms. However, it does not seem probable that this is of much economic importance in the incidence of the disease. If these cases of vegetative infection are typical, the symptoms are not likely to be confused with flag or loose smut on leaves and stem because of the tendency of the bunt strands to form spore pockets and the fact that the affected parts are greatly stunted and distorted.

SUMMARY

Chlamydospores of *Tilletia tritici* and *T. levis*, the fungi that cause bunt, were produced in the leaf and stem tissue of Prelude wheat grown in the greenhouse. The affected parts were stunted and distorted.

PHYTOPATHOLOGICAL NOTES

Armillaria Crown Rot of Strawberry. Since the writer first reported a crown rot of strawberry caused by Armillaria mellea (Vahl.) Fr. several inquiries concerning its severity have been received, and thus this occasion is taken to report briefly our experience with the disease.

The disease was first observed by the writer several years ago near Albany, Oregon. The infection areas were in the form of 3 almost perfect circles in a Marshall strawberry planting. F. D. Bailey observed a similar case in 1912 near Oregon City (see Fig. 1). These 3 circles were about 7,



Fig. 1. Circular area of strawberries killed by Armillaria mellea, near Oregon City, Oregon, June, 1912. Photo by F. D. Bailey.

1 Published as Technical Paper No. 176 with the approval of the Director of the Oregon Agricultural Experiment Station. Contribution from the Department of Botany and Plant Pathology.

11, and 24 ft. in diameter. The planting was examined rather early in April. At this time the plants in the centers of these circular areas had been removed by the grower, having died out during the winter. Plants around the margins of these infection areas were now showing considerable "yellowing" in the mesophyll of the leaves, and the plants were somewhat dwarfed. An examination showed the whole core of the crowns to be almost completely filled with a white fungous felt surrounded by a brownish, crusty layer of plant tissue, perhaps 2 mm. thick. Interspersed in the fungous felt are to be found islands of brownish host tissue. As a rule, rhizomorphs are seldom found around the crowns. Such plants totally collapse early in the summer. The infected plants were about 17 per cent of the total patch. Several instances of this kind have been observed in other locations west of the Cascade Mountains.

In almost every case where the infection areas have been localized, as in circles, the growers have reported that these are the exact spots where oak trees previously stood, and undoubtedly these were the source of the Armillaria.

Two plantings have been studied where infected plants were found singly but well distributed over the whole plantings. These were both plantings of the Marshall strawberry, one on oak-grubbed land the other on land where just previously had been grubbed miscellaneous mixture of brush and shrubby trees of hazel, Corylus californica Rose; oak, Quercus garryana Dougl.; poison oak, Rhus diversiloba Torr. and Gray; dogwood, Cornus nuttallii And.; willow, Salix scouleriana Barratt; trailing blackberry, Rubus ursinus Cham. and Schl.; etc. In the one case of oak-grubbed land there was a great deal of chips and sticks of wood plowed into the soil. Here the strawberry plants infected with Armillaria were found singly over the planting of 5 acres, constituting about 6 per cent loss of plants. In the other case about 23 per cent of the plants of a 7-acre planting showed infection, well distributed over the acreage without definite infection areas.

Notes have been recorded on 6 different plantings of the Marshall variety where Armillaria infections occurred, and 2 others have been observed. These are well distributed in the hill lands of the Willamette Valley, and one infection has been recorded for Clatsop County.

Cultures have been made from 196 plants from which 144 (or 73.5 per cent) isolations of Armillaria have resulted.—S. M. Zeller, Oregon Agricultural Experiment Station, Corvallis, Oregon.

Pineapple Disease (Thielaviopsis Ethaceticus) on Sugar Cane at Tucumán, Argentina. During the latter part of December, 1928, and the first part of January, 1929, the writer cut some P. O. J. 2725 sugar cane from one of the Tucumán Experiment Station plots for the purpose of artificially infesting it with sugar-cane borers (Diatraea saccharalis Fab.). In order to do this he made a hole for each borer with a steel punch. He then placed the canes in empty gasoline tins on clean wet sand. In March, when the canes were cut open, several were found to have a characteristic odor of pineapple and exhibited a mass of black spores that proved to be those of the pineapple disease, Thielaviopsis ethaceticus Went.

In a recent paper G. L. Fawcett¹ states, in referring to this finding of pineapple disease in the province: "An entomologist who was rearing and sending parasites of the sugar-cane borer to North America had placed some canes of the variety P. O. J. 2725 originating from one of the lots of this station (in cans) during three months under favorable conditions for the best growth of the disease; those which were examined were found to be heavily attacked by pineapple disease. All of these canes had been perforated with an instrument used previously in Jujuy for the same object; it was surely not sterilized before being used here . . . an experiment was repeated using the same cane and treatment. . . . On the first of May, 1930, three months later, they were examined and not one trace of pineapple disease was found. . . ."

This steel punch was made in Tucumán and never taken out of the Province. The writer made several trips to Jujuy, but no experiments were carried on there that required the use of such an instrument.

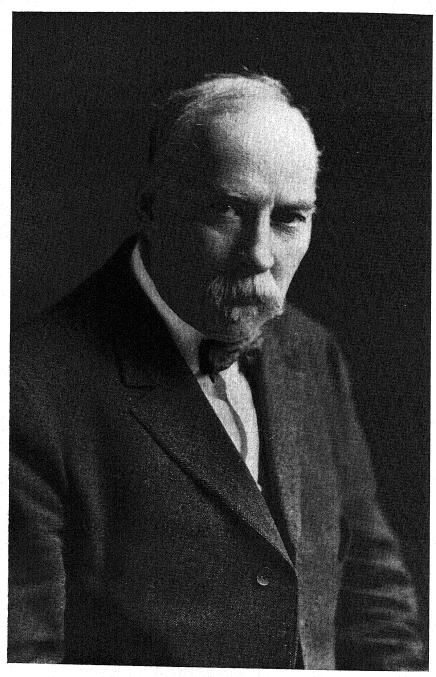
In October, 1929, some small pieces of cane then being used for rearing weevil larvae (*Acrotomopus atropunctellus* Boh.) showed signs of pineapple disease. This cane had been obtained from a field lying directly between Tafi Viejo and the experiment station at Tucumán, Argentina.

The writer then made another experiment: On October 28, he carefully sterilized in alcohol 3 jars and a cane knife and took them to the same field near Tafi Viejo. He cut short pieces of cane near the ground and placed 4 or 5 pieces in each jar. He then sealed the jars and took them back to the laboratory. On November 19 one jar showed development of black spores, and the next day another jar also showed signs of black spores. Later he opened the jars and filled them with a solution of formalin to preserve the canes. One of the jars showing the black spores was submitted to the Bureau of Plant Industry, United States Department of Agriculture,

¹ Fawcett, G. L. La putrefacción negra de la caña de azucar. Rev. Indus. y Agr. Tucumán 21: 55-59. 1931.

and the material was examined by Dr. George Sartoris, who determined it to be infected by *Thielaviopsis ethaceticus*, the cause of pineapple disease.

The methods employed in the sugar-cane-borer parasite work were such as to eliminate the possibility that the pineapple-disease infection could be brought into Tucumán on infected tools used in this work. The writer's observations and experiments have established definitely the presence of pineapple disease in the field in the Province of Tucumán, Argentina.—H. A. Jaynes, Associate Entomologist, Bureau of Entomology, U. S. Department of Agriculture.



LOUIS HERMANN PAMMEL

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LOUIS HERMANN PAMMEL 1862–1931

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The death of Louis Hermann Pammel deprived The American Phytopathological Society of one of its charter members and its membership of a dear personal friend and associate. While primarily a taxonomist, he had capacity to devote himself to many other fields and to make notable contributions to the knowledge therein.

Dr. Pammel was born at La Crosse, Wisconsin, April 19, 1862, and, after being graduated from the University of Wisconsin in 1885, he continued his studies, first at Harvard University under Doctor Farlow and then at the Shaw School of Botany, Washington University, under Doctor William Trelease. In 1889 he received his master's degree from the University of Wisconsin. He returned to St. Louis in 1896 and received his doctor's degree from Washington University in 1899.

After leaving St. Louis in 1889, Doctor Pammel was called to Iowa State College to take charge of the botanical work left by Doctor Byron Halsted. At that time the staff of the botany department consisted of one person, himself. At the time of his death, March 23, 1931, the staff had grown to include 15 members, offering 56 courses, with major work in ecology, morphology, mycology, pathology, physiology, and systematic botany. Such expansion proclaims the man.

Doctor Pammel was a man of wide acquaintanceship, having contacts with persons in far places and many diverse callings. In all his endeavors the personal side of life had for him the greatest appeal. With his students and his associates he stressed the importance of the individual and his responsibilities to others, and he always found, on his many journeyings, a host of welcoming friends.

In his science Doctor Pammel was always a pioneer, and with that spirit he entered into all of his work. He was interested in the unknown and active in carrying through investigations in new fields. Thus, if the important contributions that he made be examined, it soon becomes apparent that he wanted to be on the frontiers of his chosen field. His early work was in plant pathology, and we find him in 1889 upholding a soil fungus as the causal agent of cotton root rot at a time when the dominant note in agriculture was emphasizing the importance of chemical balance in soils

and explaining crop failures in terms of chemical deficiencies. Again, he was among the leaders in ascribing a plant disease to a bacterial organism in the case of the black rot of rutabaga when the question of bacterial diseases of plants was still controversial. Still later, he was among the first of his fellows to recognize the importance of some of the fungi imperfecti in cereal diseases, and his contributions on the Helminthosporium diseases of barley broke ground for many later studies on these fungi. In addition, Doctor Pammel made some of the earliest investigations on the use of fungicides to control the leaf spot of cherry, and he pointed out the futility of seed treatment for corn smut. These, with a continuous stream of short papers and observations on the occurrence of plant parasites, make up his contributions to pathology.

In our estimate of the man it must be remembered that plant pathology was not his main field of endeavor, for it was within the field of taxonomic botany that he felt most at home. In this sphere of influence he again showed his pioneering spirit. His encyclopedic "Poisonous Plants," his "Grasses of Iowa," and his "Weed Flora of Iowa," all were forerunners of a host of publications along very similar lines by other investigators. His last work, "Honey Plants of Iowa," brought together much data from a new point of view. One of the investigations in which he took most pleasure was his exploration of the vegetation of the Uintah Mountains, and, as he grew older, he got great enjoyment from visiting new places and comparing their floras with the one he knew so well, Iowa.

As a teacher the best criterion is doubtless the renown of the men who have sat at his feet. With Doctor Pammel such a test leaves little more to be said, for his students have reached the forefront of the ranks in all phases of botany and the enthusiasm for plants that he imparted has had a lasting influence.

Along with his teaching and his research Doctor Pammel always maintained an abiding interest in the social side of life. He was deeply religious and a leader in the work the churches were carrying on among the student bodies in our colleges. For years this work was a hobby, which he rode to a successful conclusion in the Episcopal Church at Ames as well as nationally. Another interest to which he gave much time was that of technical and honorary societies. He maintained membership in many foreign as well as American societies and served at different times as Secretary General to both Phi Kappa Phi and Gamma Sigma Delta. He was President General of the former, 1923–27. In 1919 he was Vice President of Section G of the American Association for the Advancement of Science.

Among the honors that Doctor Pammel received was the naming of a grass *Hordeum pammelii* Scribn. and Ball and of a smut *Entyloma pammelii* Hume in recognition of his work in grasses and their diseases.

Senecio pammelii Greenman was named for its discoverer. The degree of doctor of science was granted him by the University of Wisconsin in 1925.

In Iowa his most notable achievement was again in the pioneer field of conservation, and it was as the Chairman of the State Board of Conservation that he helped establish the system of State parks that has been a marked step in preserving many of the beauty spots of Iowa. Upon his retirement from the board a graceful tribute was paid to his work by naming the park area at Winterset after him.

When we look back over Doctor Pammel's life and his achievements we are reminded of the pioneers depicted by Joaquin Miller:

"Full were they
Of great endeavor. Brave and true
As stern Crusader clad in steel,
They died a-field as it was fit.
Made strong with hope, they dared to do
Achievement that a host today
Would stagger at, stand back and reel,
Defeated at the thought of it."

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THE EFFECT OF THE SUGARY GENE IN CORN ON RESISTANCE TO SEEDLING BLIGHT CAUSED BY GIBBERELLA SAUBINETII¹

P. H. SENN²

INTRODUCTION

It has been recognized during recent years that the organism Gibberella saubinetii (Mont.) Sacc. is largely contributory to seedling blight of corn in the United States. During the year 1927 the loss caused by this organism was estimated³ at 1.6 per cent of the entire crop or approximately 50,000,000 bu. of corn.

Several investigators (10, 14) report that both dent and sweet corn have many diseases in common but in nearly all instances the effects are more severe on sweet corn. These reports seem to agree with general observations made in Wisconsin, and studies were begun at the University of Wisconsin in 1927 in an attempt to determine more definitely the relative resistance of sweet corn to the seedling-blight organism, Gibberella saubinetii, compared to the resistance of nonsweet or starchy corn.

In the present investigation it was desired to determine whether sweet corn as a class, just because it is sweet corn rather than field corn, is more susceptible to seedling blight or whether the apparent difference in resistance of the two classes of varieties is due to some other factor or factors.

MATERIALS AND METHODS

Foundation stocks. The foundation material for this study was obtained from stocks of inbred and crossbred ears produced at the Wisconsin Agricultural Experiment Station at Madison. The inbred lines were represented by dent, flint, and sweet corns of locally adapted varieties. The crossbred ears were produced by crossing the sweet corns with the flint and dent varieties.

- ¹ Paper from the Department of Genetics, University of Wisconsin, No. 130. Published with the approval of the Director of the Station.
- ² Assistant Professor of Farm Crops and Genetics, University of Florida, Gainesville. The writer acknowledges his indebtedness to Dr. R. A. Brink, Professor B. D. Leith, and Dr. J. G. Dickson, all of the University of Wisconsin, for general guidance and helpful advice throughout the period of the investigation and in the preparation of the manuscript.
- ³ Plant Disease Survey. Crop losses from plant diseases in the United States in 1927. U. S. Dept. Agr., Bur. Plant Indus., Plant Disease Rptr. Sup. 64: 379. 1928. (Mimeographed).

In 1901 Correns (2) found a single genetic factor difference between the starchy and the sweet endosperm characters. The sugary character is recessive in inheritance to the nonsugary or starchy condition.

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Ears segregating for the sugary and the nonsugary kernels were needed for testing against the seedling-blight organism. To obtain these some heterozygous plants were self-pollinated, while others were back crossed to sugary plants. Both procedures resulted, of course, in ears segregating for sugary and nonsugary kernels. Segregating ears were produced also by crossing reciprocally sugary and heterozygous nonsugary plants. This resulted in kernels of the classes of similar residual genetic constitution but produced upon the 2 respective types of plants, sugary and nonsugary. These ears were used in studying the effects of the constitution of the mother plant.

The ears were produced during the summers of 1927 and 1928 at the Wisconsin Agricultural Experiment Station at Madison, Wisconsin. As the ears were harvested in the fall they were dried on hangers in a steamheated room equipped with electric fans for circulating the air.

The organism. The seedling blight dealt with in this manuscript is caused by a fungus, the conidial stage of which is known as Fusarium graminearum Schwabe, its ascigerous form being known as Gibberella saubinetii. Throughout the study, a single physiologic form of the organism was used. The organism was obtained from the Department of Plant Pathology of the University of Wisconsin under Wisconsin No. 259. Inoculations were made with 5- to 7-day-old cultures grown on potato-dextrose agar at room temperature.

Dickson (3), in his work with Gibberella, has pointed out that the rate of growth of the mycelium and the production of conidia, extend over a wide range of temperatures, from 4° to 34° C., but the most vigorous development is around 24° to 29°. Corn, however, makes its most vigorous growth at soil temperatures around 24° to 28° C. The most favorable soil temperature for the blighting of corn ranges from 8° to 20° C. Above 24° C. no blighting occurs.

Greenhouse technique. Experimental conditions modify considerably the expression of the disease symptoms. Consequently, reduction of variability in environment becomes essential if reliable comparisons are to be made. Temperature is one of the main variables. Absolute control of temperature in the greenhouse is, of course, impossible. The disease-resistance studies, however, were made in the greenhouse during the winter months when the temperature outside was low. The greenhouse temperature was automatically regulated by thermostats and a motor-driven blower so adjusted that when the greenhouse temperature exceeded a certain degree, cool air was drawn in from outside.

McIndoe (15) points out that not only should the temperature be constant throughout the experimental period but an equal distribution of heat over the greenhouse should be assured. This is especially necessary if a dependable estimation of relative resistance is desired. In addition to the blower, an electric fan was installed in the greenhouse at a point advantageous for circulating the air freely.

Soil temperatures were recorded 3 times daily, morning, noon, and evening, from thermometers placed about 1.5 in. deep in the soil. The temperature showed small variations, being highest at midday, as was to be expected. There were slight daily variations but these were within the range for the expression of seedling blight.

The inoculation test. Due to the limited number of the 2 kinds of kernels, 40 sugary and 40 nonsugary seeds from each segregating ear were usually planted in the inoculation test. Ten additional kernels of each kind were planted on the same kind of soil but uninoculated. The noninoculated sample adjoined the inoculated one with only a division board separating them. Ten other kernels from the same lots of seed were placed on a germinator bench for a study of germination percentage and relative vigor of seedlings and for observations as to the presence of seed-borne organisms. The same procedure was followed with both the nonsugary and the sugary kernels of the same ear. Just before planting, the kernels to be tested were placed in an aqueous conidial suspension of the Gibberella organism for about 10 minutes. The concentration of the suspension was usually around 750,000 conidia per cc.

The sugary and nonsugary kernels from the same ear, when being tested for relative resistance, were always planted side by side in rows 3 in. apart in the bench. Such an arrangement placed the two lots of kernels under as nearly identical conditions as it seemed possible to provide. The planting arrangement is shown in figure 1. The soil used was a half-and-half mixture of well-rotted sod and sand. After being sifted through a \frac{3}{8}-in-mesh screen, the soil was friable and in condition for removing the seedlings and for washing the roots preparatory to reading the results of the test.

The moisture content of the soil was maintained at what seemed to be the most favorable percentage for the germination and growth of the young plants.

Germination tests. Ten sugary and 10 nonsugary kernels from each segregating ear were placed on the germinator in rows side by side. Besides the germination percentage on each lot, the vigor of the seedlings was observed. Observations on the number of healthy seedlings as well as on the presence of various kinds of seed-borne organisms were likewise recorded.

The germinator used was of the limestone-sawdust type described by Hoffer and Holbert (8). The kernels were placed between sterilized cloths and kept moist by daily sprinklings. The germinator was located in a basement where the temperature differed little from that maintained in the greenhouse where the inoculation tests were run. Records were taken on the seedlings after about 14 days on the germinator.

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From the noninoculated or control bench, records were obtained on the percentage of kernels germinating, the number of healthy plants, and the number having lesions or blighting. As nearly as possible without plating, the cause of the lesions or the blighting was determined. The combined records from the control bench and the germinator served as the basis for analyzing the results of the inoculation trials.

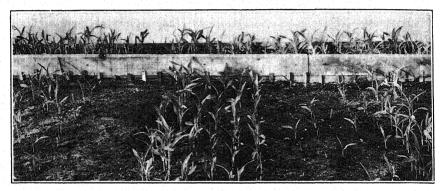


Fig. 1. As shown in the planting arrangement above, sugary and nonsugary kernels from each ear are planted side by side in rows 3 in. apart in the inoculated bench. The corresponding noninoculated or control seedlings may be seen beyond the division board. A high correlation between the reaction to the fungus of the sugary and nonsugary seedlings from a given ear is in evidence. In the middle foreground the sugary and nonsugary seedlings from 3 resistant ears are seen. At the right and left of this group both classes of seedlings are highly susceptible. It is evident that genes other than the Su-su pair may play an important rôle in determining resistance and susceptibility.

Symptoms of seedling blight. As Dickson (3) has pointed out, the symptoms of corn-seedling blight caused by Gibberella are variable, depending upon many factors, such as severity of attack, the age of the seedling, and the environmental conditions during the development of the disease. The seedling may be attacked at various stages. The regions of attack are primarily the coleorhiza and the cortex of the mesocotyl. In the case of severe attacks many of the seedlings may be killed before they reach the surface of the soil. In most cases the developing embryo is invaded and killed before either plumule or roots elongate. Some seedlings do not succumb to the attack until the 1st- or 2nd-leaf stage. The parasite invades

the cortical tissues of the young seedlings, causing them to become dwarfed and chlorotic.

Rather definite lesions usually extend from the kernel into the seminal root and adventitious roots as well as into the mesocotyl. The fungus invades the outer soft or parenchymatous tissue, which usually becomes light brown to reddish brown and water soaked in appearance. While the badly infected seedlings become yellow and wilt, many of the plants partially recover by sending out roots usually at the node above the lesion and make a fair growth.

Classification of the seedlings. Since the symptoms of seedling blight are most pronounced in the early stages of the life of the corn plant, the seedlings were grown to the 3rd- or 4th-leaf stage, in a period of about 21 days. On completion of the test the seedlings were classified into 5 groups, according to a scheme devised by Hoppe (unpublished) as follows: (1) Healthy, (2) restricted lesions, (3) endodermal resistant, (4) blighted after emergence, (5) blighted before emergence.

The "healthy" class included those plants showing no lesions.

The "restricted-lesions" class comprised the plants showing one or more small lesions either in the region where the primary root comes out of the coleorhiza or in any restricted area of the mesocotyl.

In cases where the lesion had completely encircled the mesocotyl and at the same time the seedling was showing signs of sending out secondary roots above the lesion the plants were placed in the "endodermal-resistant" class. Here resistance is exhibited through the activities of the endodermis and pericycle, preventing the fungus from entering the stele until after the seedling has had time to send out secondary roots at the node above the lesion. The young plant is thus enabled to obtain water and nutrients through these secondary roots and will often grow to maturity.

In the "blighted-after-emergence" class are placed those plants that have gotten aboveground and have begun photosynthesis but are so badly diseased that they blight.

The "blighted-before-emergence" class includes those individuals in which the pericarp bursts and the embryo begins to grow but in which the young plant is unable to push its way above the soil.

The 5 classes are represented in figure 2. While it is recognized that the classes are only relative groupings, they represent, however, an attempt at measuring the degree of resistance shown by the seedlings.

In making a comparative study involving 2 groups of material differentiated by one pair of genetic factors, such as nonsugary and sugary, it is important to reduce the effects of other factors to a minimum. The two groups of material should be made as nearly comparable as possible with the exception of the one essential pair of genes differentiating the two groups.

In order to make other environmental as well as genetic factors comparable in the two lots of seed, the pollinations were so controlled that the two types of kernels were produced on the same cob. With the two types of kernels scattered at random over the entire ear, it seemed reasonable to suppose that the differences of environment were minimized as far as is possible under the circumstances. Moreover, with this procedure the effects of the residual inheritance on resistance should be the same for both classes of seedlings.

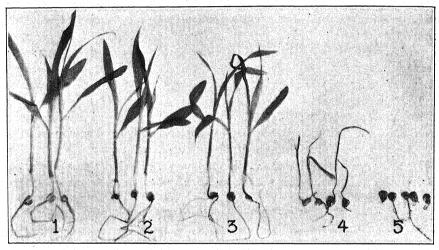


Fig. 2. Representative seedlings in the 5 classification groups: 1, Healthy; 2, restricted-lesions; 3, endodermal-resistant; 4, blighted-after-emergence; 5, blighted-before-emergence.

Index of resistance. In arriving at a figure that would represent the relative degree of resistance of a given lot of seedlings, a method was used that involves the assignment of a numerical value to each of the 5 classes, as follows:

Class			Resis	tance	value
Healthy	•••••		 	8	
Restricted-lesions		••••••	 	6	
Endodermal-resistant		•••••		4	
Blighted-after-emergence			 	2	
Blighted-before-emergenc	e	***************************************	 	0	

In arriving at the net resistance for a particular group of seedlings, the number of seedlings in each class was multiplied by the resistance value for that particular class. The sum of the products was then divided by the total number of seeds germinating. The result was taken as the average resistance of the group and is termed the "resistance index." However, this method ignores the kernels that do not form a sprout. Some kernels exhibit no growth beyond a slight break in the seed coat. This break opens an avenue of entrance for the organism. The seedling might be attacked before hardly any growth of it would be in evidence. Such seedlings would be classified as "dead" or as "not germinating," when rightfully they belong in the blighted-before-emergence class.

Some kernels were questionable as to classification. In order to give consideration to this point it was thought best to reckon the vitality of a group of kernels under inoculation by the percentage of kernels germinating on the germinator and on the control bench. When the total resistance of a group was divided by the calculated vitality of the group, the average resistance for the group of kernels tested was obtained. This method was employed in arriving at the resistance indices for the various groups of kernels tested.

For determining whether a significant difference in resistance to seedling blight was shown between the groups of sugary and the nonsugary kernels, Fisher's (7) method for arriving at the significance of a mean was employed. The resistance index of one group of kernels was paired with the resistance index of the other group of the same ear.

STATISTICAL COMPARISONS

On lot of 126 ears segregating for the two kernel characters was subjected to test. Here no consideration was given to the possible presence of any kind of seed-borne organism. In this lot it was found that the nonsugary kernels showed a greater degree of resistance to Gibberella than the sugary kernels, by an amount equal to 0.92 in terms of the resistance index. When this difference is evaluated in Fisher's tables of probability (tables of t) it is found to be statistically significant.

Seed-borne organisms. One of the outstanding things noted when reading the results on the germinator was the prevalence of seed-borne organisms on the kernels. However, such a condition does not seems to be unusual, particularly in certain seasons. Holbert and his coworkers (11) tested in 1920–21 more than 2,000 bushels of seed corn before they obtained a desired sample of 75 ears that was 100 per cent viable and nearly disease free. Moore (17) of the New Jersey station was unable to find a single sample that was entirely free from organisms.

Various pathogenic organisms were found on the kernels when reading the germinator, chief among which were Penicillium, Basisporium, and Rhizopus. Records were made of the kernel lots carrying these seed-borne organisms. The particular kind of organism present on them was noted also in so far as could be determined without plating out the individual kernels.

Preference of organisms. A study was made to see if a particular organism showed a preference on the ear for either the sugary or the non-sugary type of kernel. Twenty ears segregating in a 1:1 ratio were available for this study. These ears were used because the nonsugary and sugary kernels on each ear were approximately equal in number.

Samples of about 50 seeds each of the sugary and the nonsugary types were taken from each ear. In taking samples all the kernels from a particular area on the cob running from butt to tip were included. The ear was divided roughly into 3 sections, butt, middle, and tip. The kernels from each section were placed in a separate group on the germinator, and the nonsugary kernels were separated, of course, from the sugary.

The percentage of infection was recorded on each type of kernel in each section, and the different types of organisms observed on each of the infected kernels were also recorded. Penicillium and Rhizopus were the two most prevalent organisms. No apparent preference was shown by either of these organisms for the sugary or the nonsugary kernels.

Among the more than 2,000 kernels tested on the germinator from the 20 ears, there were only 4 kernels exhibiting an infection of Gibberella. Three of these infected kernels were nonsugary and one was sugary. Basisporium was found on 5 of the 20 ears. In every case except one the nonsugary kernels showed a slightly higher infection than did the sugary kernels. However, in only one case was the number of Basisporium-infected kernels as great as 9 on any single ear, and in this case there were 5 nonsugary and 4 sugary kernels infected with the organism.

Such results indicate that the more prevalent organisms seem to have very little, if any, preference for the one type of kernel over the other. The test also shows that the seed-borne organisms do not appear to be confined to any particular area but are scattered along the ear from butt to tip, as shown by the rather general distribution among the different sections of the ear.

Seed-borne organisms and resistance. It was not known what pathological influence upon the host any one of the seed-borne organisms might have in the presence of another fungus. Realizing this fact, it seemed necessary to determine the various organisms upon the ears and to analyze the data obtained from the kernels inoculated with Gibberella accordingly. It was desired to know if the presence of any of these organisms materially affected the relative resistance of the sugary and the nonsugary kernels to Gibberella. The basis of the ear grouping rests upon the presence or absence on the kernels of any one of the above-named seed-borne organisms. For a sta-

tistical study the ears were grouped with reference to the seed-borne organisms, as shown in table 1.

Difference in resistance. In a group consisting of 84 ears Gibberella was not observed on either the sugary or the nonsugary kernels. When inoculated with Gibberella the nonsugary seedlings exhibited a resistance index greater by 0.98 of a unit than that of the sugary seedlings. This higher resistance of the nonsugary kernels is statistically significant. Another group consisted of ears where Gibberella was observed on a few of the nonsugary kernels but not on the sugary. In this group the resistance index of the nonsugary class exceeded that of the sugary by 0.98 of a unit. There were 18 ears whose record on the germinator showed no evidence of Gibberella on the nonsugary seeds, but the organism was observed on some of the sugary kernels. Here, again, the greater resistance to Gibberella is shown by the nonsugary seedlings, the difference being 0.79 of a unit. In a 4th lot of ears involving Gibberella, where the organism was present on both the nonsugary and sugary kernels, a mean difference in resistance to Gibberella infection of 0.40 unit was found. Again, the greater resistance was in favor of the nonsugary kernels.

Another grouping was made on the basis of the presence or absence on the germinator of Penicillium. In a lot of 21 ears in which Penicillium was not observed on the germinator on either the sugary or the non-sugary kernels, the resistance of the nonsugary kernels exceeded that of the sugary by 0.68 of a unit. Another lot, composed of 19 ears, showed Penicillium on the nonsugary but not on the sugary kernels on the germinator. When the kernels were inoculated with Gibberella, the nonsugary seedlings gave the higher resistance index, the difference being 0.74 of a unit. A group of 24 ears was studied that showed no Penicillium on the nonsugary, but the organism was found to be present on some of the sugary kernels. The resistance index of the nonsugary seedlings was higher by 1.4 units than that of the sugary seedlings. Penicillium was observed on the germinator on both the nonsugary and the sugary kernels of 60 segregating ears. In this case a difference in the resistance indices of 0.88 was found, again in favor of the nonsugary seedlings.

One set of ear groupings was concerned with the presence or absence on the germinator of the organism Basisporium. One lot of 75 ears showed no evidence of Basisporium on either the sugary or the nonsugary kernels. When these ears were subjected to Gibberella the resistance index of the nonsugary class exceeded that of the sugary by 0.84 of a unit. Another group showed Basisporium present on both the nonsugary and sugary kernels on the germinator. When subjected to Gibberella inoculation a higher resistance index by 0.65 of a unit was exhibited by the nonsugary over that of the sugary class.

EarsTABLE 1.—Differences in relative resistance of nonsugary and sugary kernels of segregating ears to Gibberella saubinetii. grouped according to the presence or absence of certain seed-borne organisms

Significance of difference as determined from value of P	P = <0.01		P = < .01 P = < .01	, V	$P = \langle .02 \rangle$	P = < .04	P = < .01	P = < .01	P=<.01	P=< .02	P = < .01	F = < .01
Resistance greater by	0.92	86.	.98 .79	.40	89.	.74	1.40	88.	.84	.65	77.	88.
Greater resistance shown by	Nonsugary	Nonsugary		33	Nonsugary	3	,,	4. (3) 9	Nonsugary	•	Nonsugary	
Number of comparisons	126	84	14 18	10	21	10	24	09	75	15	72	1
Organism present on sugary (su ^a)		N_0	Yes	33	No	,,	Yes	,,,	$ m N_0$	Yes	No	Z D
Organism present on nonsugary (Su ³)	***************************************	N_0	m Yes $ m No$	Yes	m No	Yes	No	m Yes	No	Yes	No	T GS
Seed-borne organism	Not considered	Gibberella		,	Penicillium	,,	,,	3	Basisporium	,	Rhizopus	
Group No.	Ħ	c) ·	o 41	e e	9	2	∞	G.	10	7	12	

a The genetic symbol Su indicates nonsugary (starchy) kernel; su, sugary (sweet) kernel.

b From Fisher's Probability Table (table of t).

In Fisher's table of t 0.05 may be taken as the upper limit of the significance of a difference.

The organism Rhizopus was considered in connection with two lots of ears. One lot of 72 ears showed no Rhizopus on the germinator on either the nonsugary or sugary kernels. When subjected to Gibberella inoculum the nonsugary seedlings showed a higher resistance to the organism, the difference being 0.77 of a unit. The second lot, composed of 27 ears, showed Rhizopus present on both the sugary and the nonsugary kernels on the germinator. A higher resistance to Gibberella infection by 0.98 of a unit was shown by the nonsugary over the sugary class of kernels.

Ears exhibiting 100 per cent germination as well as showing healthy seedlings on the germinator and on the control bench were considered alone in a group. Of all the ears tested only 4 showed this disease-free condition. The nonsugary seedlings gave a higher resistance index than the sugary seedlings by 0.40 of a unit.

Seedling vigor. A test was made to determine whether vigor, as expressed on the germinator by amount of growth of seedling, might influence resistance to Gibberella. These results are shown in table 2. Both classes of seedlings from one group of 40 ears were rated as "strong." Upon testing these ears against the seedling-blight organism, the resistance index of the nonsugary seedlings exceeds that of the sugary seedlings by 0.62 of a unit. A similar study was made using those ears from which the sugary and the nonsugary seedlings were both rated as "weak" on the germinator. On subjecting these ears to Gibberella inoculation, a difference of 0.55 of a unit was found in the resistance indices of the two types of kernels, the difference being in favor of the nonsugary type.

Reciprocal crosses. Reciprocal crosses were made between 4 nonsugary and 4 sugary plants. Since the genetic make-up of the seeds on the reciprocally pollinated ears would, on the average, be similar, the opportunity was presented to test the effect of the parental type, sugary or nonsugary, on resistance. It was necessary, of course, to compare the non-sugary kernels from the heterozygous plant with the corresponding class from the sugary plant. The sugary kernels from the 2 types of plants likewise were compared. The results are shown in table 3.

When the nonsugary kernels from the two classes of parent plants were tested a difference in the index resistance of 0.47 of a unit was found, the greater resistance being shown by the kernels that were borne on the heterozygous nonsugary plant. Considering the sugary class in the same manner, the higher index of resistance to Gibberella infection of 0.37 of a unit was shown by the kernels produced on the heterozygous nonsugary plant. Since the number of ears in each of these comparisons was limited, the results cannot be considered conclusive. They suggest, however, that the degree of resistance to Gibberella infection appears to be but little affected by the composition of the maternal parents as such with respect to the sugary and the nonsugary genes.

TABLE 2.—Differences in relative resistance of nonsugary and sugary kernels of segregating ears to Gibberella saubinetii. Ears grouped according to the health and vigor of seedlings

Group No.	Seedling characteristics	Comparisons made between	ns made een	Number of comparisons	Greater resistance shown by	Resistance greater by	Significance of difference as determined from value of Pb
-	All seedlings healthy 100 per cent germination	Nonsugary (Sua)	Sugary (sua)	4	Nonsugary	0.40	P = < 0.40
c 0	Vigor of seedling	Nonsugary strong	Sugary	40	Nonsugary	79,	P = < .01
ന	Vigor of seedling	Nonsugary weak	Sugary weak		Nonsugary	.55	P = < .20

a The genetic symbol Su indicates nonsugary (starchy) kernel; su, sugary (sweet) kernel.

b From Fisher's Probability Table (table of t).

In Fisher's table of t, 0.05 may be taken as the upper limit of the significance of a difference.

TABL

LABLE 3.	.—Differences in	ABLE 3.—Differences in relative resistance of nonsugary and sugary kernels to Gibberella saubinetii. Kernels were produced reciprocally on heterozygous nonsugary plants	of nonsugary and sugary kernels to Gibberella saubi heterozygous nonsugary plants and on sugary plants	kernels to Gib lants and on sr	berella saubinetii. Ke ugary plants	rnels were prod	uced reciprocally on
Group No.	Type of kernels com- pared	Type of plant kernels were produced on— Comparisons made between	were produced on— de between	Number of comparisons made	Greater resistance shown by kernels produced on	Resistance greater by	Significance of dif- ference as deter- mined from value of
7 7	Nonsugary (Su) Sugary (su)	Heterozygous nonsugary (Su*) plant Heterozygous nonsugary (Su)	(Homozygous) sugary (sua) plant (Homozygous) sugary (su) plant	4 4	Heterozygous nonsugary plant Heterozygous nonsugary plant	0.47	P = 0.40 $P = 0.40$
a The b Fron In F	genetic symbol & Fisher's Proba	a The genetic symbol Su indicates nonsugary (starchy) kernel; su indicates sugary (sweet) kernel. b From Fisher's Probability Table (table of t). In Fisher's table of t , 0.05 may be taken as the upper limit of the significance of a difference.	(starchy) kernel; su t).	indicates suga significance of	ry (sweet) kernel. a difference.		

Kernel weight and resistance. A study was made to see if the weight of the kernel was correlated with the degree of resistance the seedling shows when subjected to Gibberella inoculation. It was also desired to know if such a correlation was higher in the nonsugary than in the sugary kernels.

Individual kernels were weighed to the nearest mgm. and grouped in 10 mgm. classes, the groups ranging from 80 to 370 mgm. All the kernels in a particular weight group were inoculated and planted together. The kernels from each ear were considered separately and the sugary and nonsugary kernels from the segregating ears were likewise considered as separate groups. Eighty to 100 kernels of each kind were tested from each ear. The average weights of the kernels that produced the seedlings in the various class groups were computed. The average kernel weights of each ear were arranged under the various resistance class groups. Very little, if any, correlation was found between the weight of the kernel and the degree of resistance the seedling grown from it exhibited when subjected to Gibberella inoculation. This appeared to be the case for both the sugary and the nonsugary kernels, regardless of whether they were produced on homozygous or on segregating ears. The groupings are shown in table 4.

DISCUSSION

Comparative studies between corn seedlings from sugary and nonsugary kernels with reference to their resistance to seedling blight caused by Gibberella saubinetii have been attempted in an effort to determine the effect the sugary gene exerts on resistance to the disease. By grouping the seedlings from the two kernel types into classes according to degree of infection and by the use of a resistance index, such studies are made possible.

It was not known what pathological influence any one of the seed-borne organisms concerned might have upon another when two were present together upon the same kernel. Several investigators have observed certain pathological influences among other organisms. Vasudeva (22), working with Botrytis allii and Fusarium fructigenum an apple tissue, found that the attack was much more reduced when both organisms were present than when only one was present. He states that the probabilities are that the effect is simply one of interference of one fungus with the growth of the other. Similar effects have been reported by other workers. Thus, Porter (19) claims that the attack of wheat by Helminthosporium and of flax by F. lini was reduced by the antagonistic action of a bacterium upon the fungus. Millard and Taylor (16) found that the presence of saprophytic species of Actinomyces markedly reduces the attack of potash-scab organism.

Various seed-borne organisms were found present on some of the corn kernels when examined on the germinator. Realizing this fact, it seemed

TABLE 4.—Correlation—Weight of kernel and degree of resistance of the seedling to Gibberella saubinetiis

			Classes show	Classes showing graded scale of resistance (0-8)	f resistance (0-8)	
Ear number	Kernel type	Healthy seed- lings Resistance value	Mesocotyl- lesioned Resistance value	Stelar-resistant Resistance value (4)	Blighted-after- emergence Resistance value	Blighted before- emergence Resistance value
		(8) Average weight of kernels	(6) Average weight of kernels	Average weight of kernels	(2) Average weight of kernels	Average weight of kernels
Segregating Ears	g Ears					
214-7	100 nonsugary	291	277	287	281	271
	80 sugary	252	259	263	500	253
214–26	100 nonsugary		240	239	233	237
	80 sugary		218	220	509	213
217-14	100 nonsugary		334	345	325	316
	100 sugary	230	310		250	271
$197-30 \times 207-21$	100 nonsugary		210	194	197	198
	100 sugary	170	184	174	184	187
$215-20 \times 208-9$	100 nonsugary	240	222	219	225	226
	100 sugary	202	205	210	202	206
$220-14 \times 211-25$	100 nonsugary	222	214	218	217	221
	100 sugary	210	212	207	201	199
Nonsuaarn	. Ears					
191–33		218	208	208	187	210
	100 nonsugary	244	229	239	240	235
201-43	100 nonsugary	210	200	215	208	187
Car gama Ware	77.00					
209-1	80 sugary	170	233	238	226	230
910-9	100 sugary		223		218	227
	100 sugary	120	117	118	108	121
a The ferma in the re	the resistance classes are the average weights of the kernels that produced the seedlings in those classes.	the average weight	s of the kernels th	at produced the se	edlings in those cl	asses. Weights are

advisable to determine the various organisms on the ears and to analyze the data obtained from the kernels inoculated with Gibberella, accordingly.

When Gibberella was not observed as a seed-borne organism on either the sugary or nonsugary kernels, the nonsugary seedlings exhibited a resistance index greater by 0.98 of a unit than that of the sugary seedlings. Eighty-four ears were available for this comparison. The presence of the organism on the nonsugary but not on the sugary seeds resulted in no change in the average difference in resistance of the two classes of seedlings. When the organism was observed on the sugary but not on the nonsugary kernels on the germinator the greater resistance of the nonsugary over the sugary was slightly reduced, namely, to 0.79 of a unit. When the organism was present on both types the greater resistance of the nonsugary over the sugary was further reduced, this time to 0.40 of a unit. While the greater resistance index is again shown by the nonsugary kernels, this difference is possibly not statistically significant. However, this decrease in resistance of the nonsugary as compared with the sugary, when the organism was present on both types of kernels of the same ear, does not necessarily mean that the decrease is due to the effect of the seed-borne organism. The presence of Gibberella on the seed might be coincident with other factors that prevent the seedlings from the nonsugary and sugary kernels from giving expression to an inherent difference in resistance to seedling blight, even though such a difference might exist between the two types of kernels. It was found that, regardless of the presence or absence of Penicillium from the kernels of the segregating ears, in every case the resistance to Gibberella infection was higher in the nonsugary than in the sugary kernels. differences in resistance of the two classes of seedlings ranged from 0.68 to 1.40 units.

It was found that when Basisporium was absent from both kernel types on the germinator, the nonsugary kernels showed a greater degree of resistance to Giberella infection by 0.84 of a unit. When Basisporium was present on both kernel types, the increased resistance of the nonsugary over the sugary was found to be slightly lower, 0.65 of a unit. With 15 comparisons, however, the higher resistance of the nonsugary kernels is probably significant. The decrease in resistance suggests that there might possibly be some influence exerted by Basisporium as a seed-borne organism. If such be the case in the groups of ears studied, the influence of Basisporium was not exerted to the point where an inherent difference in resistance of the nonsugary and sugary kernels to seedling blight was prevented from expressing itself.

Seventy-two ears showed no Rhizopus on the germinator on either type of kernel. When these were subjected to Gibberella inoculation, the non-

sugary seedlings exhibited a greater degree of resistance over the sugary by 0.77 of a unit. Another lot of 27 ears showed the organism present on both the nonsugary and sugary kernels. Under inoculation the nonsugary showed a greater index of resistance over the sugary by 0.98 of a unit. In view of these differences, Rhizopus seems to have no apparent effect upon the resistance of the 2 types of kernels to the seedling-blight organism.

In a group of 126 ears where no consideration was given to seed-borne organisms, a comparison was made between the resistance of the seedlings from the sugary and nonsugary kernels, to Gibberella infection. The nonsugary seedlings were found to exhibit a resistance index greater by 0.92 of a unit than that of the sugary seedlings. The difference is significant.

From the data presented regarding the influence of certain seed-borne organisms on resistance to seedling blight it is evident that a higher degree of resistance was shown by the nonsugary kernels in every group. In only one case was the difference not large enough to be considered significant. This was found where Gibberella was present on both the nonsugary and sugary kernels of the segregating ears. With 10 comparisons the resistance of the nonsugary was greater by 0.40 of a unit than that of the sugary. While this difference cannot be considered statistically significant, the greater resistance was still in favor of the nonsugary kernels.

Four segregating ears showed 100 per cent germination and all the seed-lings were healthy on the control bench. When these were tested the non-sugary kernels showed a slightly higher index of resistance, greater by 0.40 of a unit. This small difference in resistance, obtained with 4 comparisons, indicates that the difference may not be significant. A possible explanation for the small difference with these apparently healthy ears is that the plants are inherently resistant, due to the presence of other genetic factors.

In the study of resistance where vigor of seedling was concerned, it was found that when the seedlings from the nonsugary and sugary kernels were rated as "strong" on the germinator, the nonsugary exceeded the sugary in resistance by 0.62 of a unit. This difference is based on 40 comparisons. When both types of kernels on the germinator were rated as "weak," the nonsugary again showed the greater degree of resistance; however, the difference was found to be reduced to 0.56 of a unit. In the group where the seedlings were rated as strong the greater resistance of the nonsugary was considered clearly significant, whereas in the group where the rating was weak the difference was reduced to a point that cannot be considered statistically significant. McIndoe (15) made observations on seedling vigor and resistance to Gibberella and found that in certain vigorous lines of corn factors for resistance were almost absent and no opportunity for variability existed.

The results obtained from tests with Gibberella do not indicate that the presence of the various seed-borne organisms found on the kernels materially affects the resistance of the nonsugary and sugary kernels to Gibberella infection. In every group of segregating ears studied the greater degree of resistance was shown by the nonsugary kernels. Only 3 groupings of ears showed differences in resistance indices that could not be considered statistically significant. In one of these both the sugary and nonsugary kernels were rated as weak on the germinator. In another group, composed of 10 ears, an examination of the records indicated that 80 per cent of the ears showed a seedling-vigor rating of weak and the other 20 per cent a rating of only "medium strong." It seems logical to conclude that, even though an inherent difference in degree of resistance should exist between the nonsugary and sugary kernels, the expression of such a difference might easily be covered up by factors making for a weakened condition of the seedlings. A third group that showed a small difference in resistance between the 2 classes of kernels was composed of ears that gave not only 100 per cent germination but of which all the seedlings were healthy on the control bench. This grouping is shown in table 2, group 1. Only 4 such ears were found and the difference of 0.40 in favor of the nonsugary kernels cannot be considered significant. It is possible that these ears possess other factors in their residual inheritance that are responsible for the slight difference in resistance between the nonsugary and sugary kernels.

When starchy and sweet corns are studied a number of differences are found to exist between them, some of which can be seen under a wide range of environmental influences, while others can be observed only under a particular set of environmental conditions. It has been shown that the expression of resistance of the nonsugary and sugary kernels to Gibberella seedling blight is dependent upon certain temperature relationships. Environmental factors are known to influence materially seedling growth and reactions. Kidd and West (13) have indicated that the growth of a plant directly produced from seed is a function of the early development of the seedling and have designated this relation as "physiological predetermination" of the seed or seedling. These workers found evidence that crops are directly influenced by the physiological condition of the seed and of the seedling, independently of their hereditary qualities. Environmental conditions that affect the parent may also markedly influence the subsequent course of development of the seed produced. The effects that become visible in one generation may have to be traced back to the external conditions that have operated in a previous generation.

A marked relationship between the environment during the period of maturation and the subsequent growth responses of the seedling has been pointed out by Dickson and his coworkers (5). A strain of corn grown under an environment favorable for complete maturity produced ears resistant to seedling blight. The same strain, grown under unfavorable conditions for maturity, produced ears highly susceptible to blight. The genetic composition of the line apparently was not disturbed, but rather the expression of the characters. Under the two environmental conditions there were little differences in endosperm composition, but marked changes were found in the 2 embryos. Russell (20) reports similar responses with a strain of spring wheat.

Holbert and Burlison (9), in a study of cold resistance and susceptibility in inbred, crossbred, and open-pollinated strains of dent corn, found wide variations existing in resistance to cold injury as the corn approached maturity. Seed matured on plants high in cold resistance produced plants more resistant to Gibberella seedling blight than did seed gathered at the same time from corresponding plants previously injured by cold. According to Hoppe (12), some selfed strains of corn always show a high degree of resistance to Gibberella under a given range of environmental conditions, while other strains are highly susceptible. Strains were isolated that showed a consistent behavior at a given temperature. Eckerson and Dickson (6) have reported that seedlings grown at high soil temperatures are high in available carbohydrates and are low in available nitrogen. The cell walls are composed of pectic materials, cellulose being absent until photosynthesis begins. The parasite penetrates the walls of pectic materials apparently with little difficulty, whereas it penetrates the cellulose walls slowly. These workers state that these differences apparently explain in part the variation in susceptibility to Gibberella seedling blight. Later Dickson, Eckerson, and Link (4) added that, due to an abundance of sugar and dextrins available in the corn embryo at high temperatures, a carbohydrate reserve exists for building thicker and more resistant cell walls.

Through microchemical methods Toole (21) followed some of the histological and chemical changes taking place as germination progresses in the corn kernel. He concluded that the embryonic corn plant utilizes its own stored food for its initial growth before any change of food transfer takes place in the endosperm. He worked with dent corn. Similar studies on sweet corn should offer valuable data for a comparative study of the changes taking place in dent corn.

The ripening processes were studied by Appleman and Eaton (1), who state the sweet corn is considered ripe when the growth of the kernel ceases and the chemical changes have nearly attained equilibrium. They point out that the ripening process, being either chemical or dependent upon chemical processes, is influenced by the temperature prevailing during the ripening period.

Some observations at Madison, Wisconsin, upon segregating ears have suggested a differential rate of maturity of the sweet and starchy kernels upon the same ear. The temperature in a particular locality during the period of maturation might be such as to cause the metabolic processes to proceed in the starchy corn at a different rate from that in the sugary kernels. Should the rate of maturity in the sweet corn be lower than that in the starchy, a difference in degree of maturity at harvest might mean a decided difference in quality and in quantity of the reserve materials stored in the starchy and in the sugary kernels. Should the stored food in the kernel be of such a nature as not to promote favorable growth in the young seedling, the latter would likely be less fortified against the attacks of parasitic organisms.

The work of Pearson (18) has shown that corn seedlings may be attacked by Gibberella in the early period of germination. Should the rate of maturity be lower in the sugary than in the nonsugary kernels and the temperatures obtaining during the maturation period cause the sugary kernels to have an unbalanced food reserve at harvest time, the sugary kernels might be less fitted to resist the attacks of the organism.

The investigations reported in this paper show that a relatively greater degree of resistance to Gibberella seedling blight is exhibited by the nonsugary as compared with the sugary seedlings, regardless of the presence of certain seed-borne organisms. While starchy and sugary corns are known to differ in regard to endosperm reserves by a single genetic factor. there may also be a wide variation in composition with respect to other genetic factors. These factors are probably responsible to a considerable extent for the wide variations in resistance to seedling blight sometimes exhibited among different strains of the same type of corn. Their presence might explain in part the variations among the kernels of the same ear. McIndoe (15) studied the inheritance of the reaction of corn to Gibberella seedling blight. His work did not involve sweet corn but included 3 varieties of dent. His results led him to state that the inheritance of resistance is quantitative in nature and conditioned by multiple factors. Strains of corn differing widely in resistance to Gibberella were involved in his work. He further suggests that the number of genetic factors involving resistance is perhaps relatively few, since he was able to recover in the F, generation highly resistant and susceptible lines from crosses involving lines that differed widely in resistance.

If resistance to Gibberella seedling blight is conditioned by multiple factors, it is probable that the sugary gene differentiating the nonsugary and sugary kernels is one of the number of factors responsible for the difference in degree of resistance of the two types of kernels to the seedlingblight disease. Resistance being dependent upon multiple factors, wide variations in resistance are to be expected in various strains of corn. However, since the sugary gene tends to bring about a lowered resistance, one cannot hope to obtain in sweet corn the high degree of resistance that is possible among the starchy corns.

SUMMARY

- 1. In an attempt to study the effect of the sugary gene in corn on resistance to seedling blight caused by *Gibberella saubinetii*, comparative tests were made between the sweet and starchy kernels produced on the same segregating ear.
- 2. The segregating ears were grouped according to the presence or absence of 4 commonly found organisms on the seed: Gibberella, Penicillium, Rhizopus, and Basisporium. The effects of these seed-borne organisms were studied to see if the presence of the fungi would materially affect the degree of resistance of the kernels to seedling blight.
- 3. Very little, if any, preference appeared to be exhibited by any of the 4 seed-borne organisms for the sugary or nonsugary kernels, one over the other.
- 4. In every group of segregating ears, the higher index of resistance to seedling blight was shown by the nonsugary kernels. In only one case was the difference not great enough to be considered statistically significant. Such a case was found where the seed-borne organism, Gibberella, was present on both the nonsugary and the sugary kernels of the segregating ears.
- 5. In a group of 4 ears showing 100 per cent germination and all healthy seedlings on germinator, very little difference in resistance was found between the nonsugary and the sugary kernels. A possible explanation is that other genetic factors account for the small difference in resistance.
- 6. When the sugary and nonsugary kernels on the germinator produced seedlings that were rated as strong a significantly greater degree of resistance to Gibberella infection was shown by the nonsugary kernels. When both types of seedlings were rated as weak the resistance index of the nonsugary was not sufficiently higher than that of the sugary to be considered significant.
- 7. Very little, if any, correlation was found to exist between the weight of the kernel and the degree of resistance the seedling grown from it exhibited when subjected to Gibberella infection. This appears to be the case for both the nonsugary and the sugary kernels, regardless of whether they are produced on homozygous nonsugary or on segregating ears.
- 8. It is apparent from the results obtained that the sugary gene, which differentiates the sugary and nonsugary kernels, is also a factor responsible

for the differential resistance exhibited between the 2 kernel types to seed-ling-blight disease.

9. The sugary gene in itself lowers resistance to seedling blight and hence is a limiting factor in the production of a sweet corn possessing the same high degree of resistance found in certain strains of starchy corn.

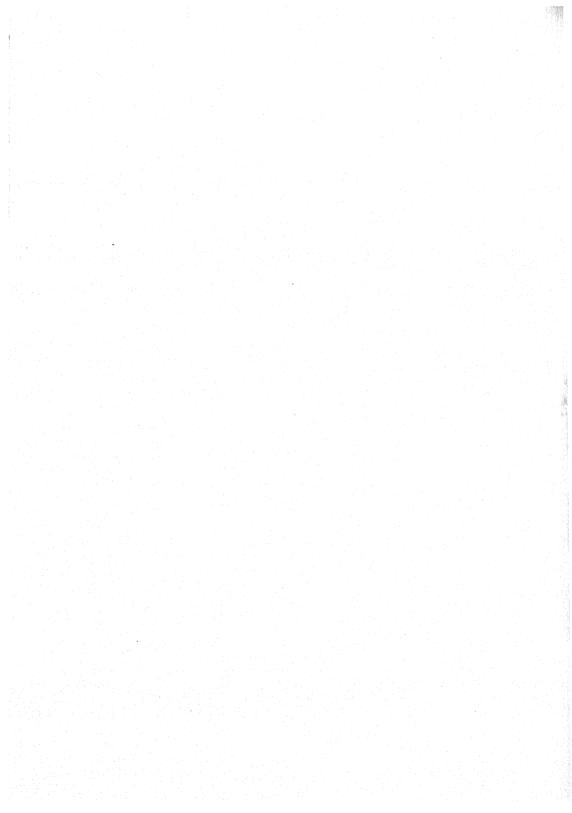
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CARBOHYDRATE FERMENTATION BY CERTAIN CLOSELY RELATED SPECIES IN THE GENUS PHYTOMONAS

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The present work was begun with the purpose of differentiating between the 3 bacterial pathogens, *Phytomonas phaseoli* (E. F. Sm.) Bergey et al., *Phyt. phaseoli* var. sojense, and *Phyt. phaseoli* var. fuscans by their ability to ferment various carbohydrates. The first 2 pathogens have never been separated in pure culture on any of their biochemical reactions, and the last-named variety is distinguished from the other 2 only by its ability to ferment the amino-acid tyrosine. The investigation, after it was begun, was expanded to include 6 other closely related species that the writer, in his article in this journal for January, 1930, had placed in what he called the campestre group in the genus Phytomonas.

Certain reasons made it desirable to study the carbohydrate fermentation of this group of organisms. In ordinary media, especially those containing beef extract, peptone, or other protein digests, these species produce a strong alkali. This characteristic has interfered in the past with correctly interpreting the results of carbohydrate fermentation with the ordinary methods used. These methods, in the main, have consisted in growing the bacteria in beef-extract-peptone broth to which the carbon source has been added. If an acid or gas is produced, the carbohydrate is considered to have been fermented, but, if the hydrogen-ion concentration of the medium remains stationary or moves to the alkaline side of neutrality, a negative reaction is recorded. The members of the campestre group, however, produce a strong alkali and immediately neutralize any acid that is formed, and many of the carbohydrates that in reality are fermented have not been so recorded. This has resulted in inconsistencies in the description of certain of the pathogens. These inconsistencies, however, in many cases may be readily discerned. For instance, a number of the species have been described as fermenting starch and not fermenting any of the monosaccharides, which is contrary to the so-called theory of carbohydrate gradients. Glucose, even, has shown no signs of being fermented, and, according to Kendall (4), "no authentic instance has been recorded in which a microbe utilizes any carbohydrate for energy that will not utilize glucose."

Furthermore, since such characteristics as indol and hydrogen sulphide production, gelatin liquefaction, nitrate reduction, and the morphology of the species are identical, one must turn to carbohydrate fermentation to separate the species in culture. For this reason the data should be accurate and only reliable methods should be employed.

PATHOGENS USED IN THESE EXPERIMENTS

Nine species and varieties of the genus Phytomonas were tested in this investigation for their ability to ferment various carbohydrates. In certain cases duplicate strains were used. As far as the writer is aware, no extensive and careful work has been conducted on the fermenting ability of these organisms with the exception of the work of Lewis (5) on the species Phyt. malvacearum.

The pathogens used in the present comparative study were as follows and, unless otherwise stated, were isolated by the writer. Duplicate strains when used also are recorded.

Phytomonas campestris (Pammel) Bergey et al. isolated August, 1929, from cauliflower leaves.

Phyt. juglandis (Pierce) Bergey et al. isolated by P. W. Miller and received from H. P. Barss, March 14, 1930.

Phyt. malvacearum E. F. Sm., Bergey et al. isolated 1923 and received from the Bureau of Plant Industry, March, 1930.

Phyt. malvacearum isolated October, 1931, from diseased cotton leaves received from South Carolina.

Phyt phaseoli isolated October, 1927, from bean pods collected in Switzerland.

Phyt. phaseoli isolated April, 1930, from bean seed collected at Clifton Springs, New York, in 1923.

Phyt. phaseoli var. fuscans isolated December, 1924, from bean leaves collected in Switzerland.

Phyt. phaseoli var. fuscans isolated October, 1927, from bean pods collected in Switzerland.

Phyt. phaseoli var. sojense (2 cultures) received from Florence Hedges, March, 1930.

Phyt. vascularum (Cobb) Bergey et al. isolated 1923 and received from the Bureau of Plant Industry, March, 1930.

Phyt. vesicatoria (Doidge) Bergey et al. isolated from diseased tomato fruits at Ithaca, N. Y., August, 1929.

Phyt. vitians (Brown) Bergey et al. isolated 1917 and received from the Bureau of Plant Industry, March, 1930.

Other species and varieties belonging to the campestre group, but, for various reasons, not included in the present investigation, are Phytomonas campestris var. armoraciae (McCulloch) Bergey et al., Phyt. citri (Hasse) Bergey et al., Phyt. cucurbitae (Bryan) Bergey et al., Phyt. papavericola, and Phyt. vesicatoria var. raphani (White). This list, no doubt, does not include all the members of the group, but meager and inadequate descriptions of many species make their placing difficult.

The 9 species and varieties used in the present fermentation studies, when grown in pure culture, were surprisingly similar in appearance. On beef-extract agar (pH 7.0) growth was good, filiform, and amber yellow to primuline yellow. On an agar medium containing dextrose, sucrose, and certain other sugars, a large amount of gum was produced giving the growth a syrupy appearance. This gum production in culture possibly gives a clue to the so-called zoogloea found in nature. Phytomonas vitians, alone, did not possess this characteristic. With the exception of this dissimilarity, however, the differences in appearance between any of the species and varieties were never greater than the differences noted between the 2 strains of Phyt. phaseoli used in the experiments.

Before conducting the fermentation experiments the pathogenicity of each organism was proved with the exception of *Phytomonas juglandis* and *Phyt. vitians*. With the former it was difficult under the conditions to obtain growing walnut trees of susceptible varieties. Lettuce, however, was inoculated with *Phyt. vitians*, but infection did not take place. Either the conditions were unfavorable, or the pathogen had become nonvirulent from many years' growth in culture.

METHODS

To determine the fermentation ability of these 9 bacterial pathogens a synthetic peptone-free base was used to which the various carbohydrates were added. It was fortunate in this respect that none of the bacteria being studied required an organic source of nitrogen but could utilize an ammonium salt. Two different basic media were employed and contained no amino acid that could be converted into ammonia. When the salts of organic acids were being tested, the medium and methods recommended by Ayers, Rupp, and Johnson (1) for alkali-forming bacteria were followed. In this case only 0.15 per cent of the salt was added to a synthetic liquid base. With the sugars, alcohols, and glucocides a slightly modified formula of the above medium was used and is the one recommended in the Manual of Methods of Pure Culture Study of Bacteria issued by the Society of American Bacteriologists (7). One per cent of sugar or other carbon source was added to the synthetic base and used as a solid medium with the addition of agar. In both cases the medium was adjusted to approximately pH 7 by the addition of sodium hydroxide, and brom-cresol purple was added as an indicator. All media, unless otherwise stated, were sterilized at 15-lbs. pressure for 18 minutes.

The ability of each organism to ferment the following carbohydrates was tested in media made in the above manner: dextrose, levulose, galactose, arabinose, xylose, rhamnose, lactose, maltose, sucrose, raffinose, dulcitol,

glycerol, mannitol, salicin, and the sodium salts of acetic, benzoic, citric, formic, malic, salicylic, succinic, and tartaric acids.

Since the sugars, levulose, lactose, maltose, sucrose, and raffinose have a tendency either to carbonize or to break down into monosaccharides when heated, a liquid medium also was prepared in which these sugars were sterilized by filtration. In this case a 10 per cent solution of the sugar was filtered through a Berkefeld filter and 1 cc. of the solution was added to tubes containing approximately 9 cc. of the sterilized basic medium. Such media were incubated for several days at 27° C. to insure their sterility before using.

In determining the hydrolysis of starch, the starch-agar-iodine method was used. Since it frequently is difficult to obtain soluble starch free from impurities, this direct method is more satisfactory than the indirect method of testing for acid production. Similarly, a direct method was employed in testing for the hydrolysis of cellulose. Strips of filter paper were added to test-tubes that in one series contained approximately 5 cc. of Dunham's solution and in another series contained the modified Ayers, Rupp, and Johnson solution.

The organisms to be tested were grown first on beef-extract agar for approximately 48 hours and then transferred to the carbohydrate media. Three tubes of each medium were used and were incubated at 27° C.

Since, with the exception of the starch and cellulose media, the only source of energy for the bacteria was the added carbohydrate, fermentation could be determined by growth alone. Fermentation, however, was always accompanied by a change of hydrogen-ion concentration. When any of the sugars, alcohols, or glucosides were fermented, the production of an acid soon changed the color of the medium from purple to yellow. With the organic acid salts, however, there was a rise in pH, showing a considerable production of alkali. According to Ayers, Rupp, and Johnson (1), these salts are fermented into carbonates.

Diastase production was not considered positive unless there was a definite visible zone about the streaks in the plate when the iodine solution was added. A slight decomposition of starch, visible only by the aid of the microscope, might be due to an acid hydrolysis and has been disregarded. Likewise, only a visible decomposition of the filter paper has been considered a positive hydrolysis of cellulose.

RESULTS OF FERMENTATION STUDIES

The data gathered from these fermentation studies have been arranged in table 1. In the majority of cases the ability of the organism to ferment a sugar or not was clear-cut, and there could be no doubt as to the inter-

TABLE 1,-Showing the carbohydrates fermented by 8 species and varieties of a closely related group in the genus Phytomonas

Tartaric acid		i	1	1	1		1	1	I,	1	ı	1
Succinic acid	-	+ -	+	+	+		+		+	+	+	+
Salicylic acid		ı	I	I	ī		i		ı	1	1	ı
Malic acid	-	 - -	+	+	+		+		+	+	+	+
Formic acid		I	1	ı	ı		i		ľ	ı	ı	ı
Citric acid	-	+ -	+	+	+		+		+	+	+	+
Benzoic acid		1	1	1,	1		ı		1	1	ı	ı
Acetic acid	-	+, .	+	+	+		+		+	+	+	+
Cellulose		1	1	ı	ı		1		1	ı	1	1
Starch		+ .	+	+	+		+		+	+	+	1
Salicin		i	i	ı	1		1		1	1	ı	1
[otinnsM		+ -	+	ı	-1		+		+	1	+	1
Glycerol	_	+ -	+	+	+		+		+	1	+	+
Dulcitol	-	ı	1	1	ļ		1		1	1	ı	1
Raffinose		+	+	+	+		+		+	+	+	+
Sucrose		+	+-	+	+		+		+	+	+	+
Maltose		+	-	+	+		1		+	I	+	1
Lactose		1	+	+	+		+		+	1	e»	+
Кһатоѕе		ı	1	1	1		1		1	1	i	ı
Aylose	-	+	+	+	+		+		+	+	+	+
esonidarA	-	+	+	ı	+		+		+	-1	+	+
Galactose		+	+	+	+		+		Ŧ	+	+	+
Levulose	-	+	+	+	+		+		+	+	+	+
Dextrose		+-	+	+	+		+		+	+	+	+
	ytomonas	ampestre	nglandis	malvacearum	haseoli	haseoli	var. fuscans	haseoli	var. sojense	ascularum	resicatoria	itians

a A plus sign means fermentation; a minus sign, no fermentation.

pretation. A few exceptions, however, were noted and are here presented. In media to which rhamnose, dulcitol, and salicin had been added, all organisms gave a trace of growth. After 2 months, however the hydrogen-ion concentration in these cultures had not changed appreciably, and it was considered that these 3 carbohydrates contained a slight quantity of impurities that permitted growth.

In considering the sugars that were filtered it should be stated that there existed no differences between them and the heated sugars with the exception of lactose and maltose. Phytomonas campestre and Phyt. vascularum grew and produced an acid in the heated lactose, but in the filtered sugar Phyt. vascularum did not grow, and only in several instances was there any growth of Phyt. campestre. In the latter case growth occurred 2 or 3 weeks after the organisms had been transferred to the tubes. From the table it may be seen that 3 of the organisms do not ferment maltose either in the heated or filtered condition. Phytomonas malvacearum, however, fermented filtered maltose but did not at all or only slightly ferment the heated sugar. On first thought this behavior appears extraordinary, but Lewis (6) has shown that this species is very sensitive to overheated sugars.

In a few cases the present study in carbohydrate fermentation does not agree with certain previous work. Negative results where peptone has been used as a base naturally have not been considered. Elliott (2) states that *Phytomons campestre* does not ferment maltose but that lactose is fermented. In the present investigations, the reverse was found to be true. The use of heated and filtered sugars might have something to do with this discrepancy or various physiologic races of the pathogen may exist.

Phytomonas malvacearum varies from the description given by Lewis (5) in that arabinose was fermented by one of the strains studied. The fermentation was slow in the liquid medium but very good on the agar medium. This characteristic probably is a variation from the normal since it occurred in only one strain of the organism in this investigation and was not found by Lewis in any of the 14 strains he studied.

In examining table 1 it may be seen that certain of the pathogens can be differentiated by their ability to ferment carbohydrates, especially sugars and alcohols. The glucocides, the polysaccharides, and the salts of the 8 organic acids were of little help. With one exception they were utilized either by all the pathogens or by none.

Phytomonas phaseoli var. fuscans, Phyt. vascularum, and Phyt. vitians are separated from the other members of the group by their inability to ferment maltose. Of these organisms Phyt. vitians alone does not hydrolyze starch. This characteristic and the fact that the organism does not produce

gum in culture separate it rather sharply from the remainder of the group. *Phytomonas vascularum* ferments relatively few carbohydrates, which distinguishes it; especially noticeable in this respect is its inability to utilize arabinose, lactose, and glycerol.

Among the 6 maltose fermenters it is of special interest to note that Phytomonas phaseoli var. sojense can be differentiated from Phyt. phaseoli by its ability to utilize the alcohol, mannitol. This test was repeated several times with 2 different strains of each pathogen, and it appears to be a clear-cut distinction. Phytomonas campestre, Phyt. juglandis, Phyt. phaseoli var. sojense, and Phyt. vesicatoria are not so easily separated. Phytomonas campestre usually does not ferment lactose, and this characteristic would separate it from the remaining maltose-fermenting pathogens. The test, however, is a delicate one, and further work should be done on a large number of strains of the pathogen before accepting this as a definite means of separation. Phytomonas malvacearum can be separated from Phyt. phaseoli only by its inability to ferment arabinose, and here certain anomalies might exist. These 2 pathogens are distinct from the remaining maltose fermenters in their inability to utilize mannitol.

A further separation of the group on the basis of the carbohydrates used is not possible. While a definite step has been made in the study of the carbohydrate fermentation of this group and the separation of the species, the problem is by no means solved. More intensive work on the individual species in which many strains are employed is needed together with further comparative studies with other carbohydrates. It is clear, nevertheless, that culturally the pathogens are very closely related, and, no doubt, if their relationship had been appreciated at the time each pathogen was described, there would have been but one species with many varieties. That they should be reduced to varieties now, however, is by no means desirable since the names are so well established in literature.

Alkali Production. According to Elliott (2), in the preface to her manual, the alkali produced by this group is ammonia. On the other hand, Ayers, Rupp, and Johnson (1) have stated that certain bacteria produce quantities of carbonates and bicarbonates from the fermentation of organic acids and thus may neutralize any acid produced from sugars or other common carbohydrates.

Certain experiments, therefore, were conducted to determine, if possible, the nature of this alkali and from what source it was formed. First, a comparative study was made of dextrose fermentation in the synthetic medium used above and in a beef-extract-peptone base. In the former case, all the bacteria turned the brom cresol purple indicator to a distinct yellow in approximately 48 hours. In the latter case where Andrade's

indicator was used instead of the brom cresol purple, a good growth was obtained within 24 hours, but at no time did the medium turn red to indicate production of acid. It was evident that a neutralization of the acid formed from the sugar was taking place.

Tests then were made for ammonia following the method advocated by Hanson (3). The bacteria were grown on Hucker's agar and in all cases there was a strong ammonium reaction. Tests also were made with the various organisms in standard beef-extract bouillon (pH 7.25), in a 0.5 per cent peptone broth (pH 7.20), and in a 0.3 per cent beef-extract broth (pH 7.25), and again ammonia was formed in all the tubes. In the peptone broth, after 48 hours' growth of the bacteria, the alkalinity rose from an initial pH 7.20 to a minimum pH 7.32 and a maximum pH 7.50. This rise probably was due entirely to the ammonia production, since the intensity of the color in the reaction varied with the pH of the culture medium. A continued production of this amount of ammonia for several days, without doubt, would neutralize any acid produced from sugar fermentation and render worthless a color indicator.

While the ammonia doubtless plays a large part in neutralizing acids produced from carbohydrate fermentation, in the presence of organic nitrogen, the alkali formed in the fermentation of organic acids should not be overlooked. Ayers, Rupp, and Johnson (1) are of the opinion that this is the chief source of alkali produced by a group of bacteria isolated from milk. They were able to show that the carbonates produced by the fermentation of the citric acid in milk were sufficient to neutralize the acid produced in the fermentation of milk sugars. The citric acid was broken down into carbonates. In the present investigation it is shown that these plant pathogens were able to ferment the salts of acetic, citric, malic, and succinic acids. Doubtless other acids exist that they are able to utilize. A very small amount of the salt was added to the media, 0.15 per cent to be exact; yet considerable alkali was produced upon fermentation. In the acetic acid medium the alkalinity within a week rose from a pH 7.00 to between pH 7.10 and pH 8.27, according to the species or culture tested. In the citric acid medium the rise in the same length of time was from an initial pH 6.90 to between pH 7.00 and pH 7.55; in the malic acid medium the rise was from an initial pH 6.80 to between pH 7.60 and pH 7.80; and in the succinic acid medium the rise was from an initial pH 6.71 to between pH 6.81 and pH 7.61.

It may be seen from these experiments that considerable alkali is formed from the organic acids in a solution of only 0.15 per cent. Whether or not the salts of these acids are present in beef extract and to what extent they are present in such media have not been determined by the writer. Also,

whether or not the organic acids that are the result of sugar fermentation are the end-products of the reaction or are utilized further is not known. This phase of the subject should be investigated before one concludes that the neutralizing alkali in the sugar-broth cultures is due to something more than ammonia.

SUMMARY

The fermenting ability of 9 species and varieties in the genus Phytomonas has been investigated. Twenty-four different carbohydrates were used.

It has been pointed out that accurate results are not to be expected when the sugar or other carbon source to be tested is added to beef-extract-peptone broth. In this medium the pathogens produce a strong alkali, which neutralizes any acid formed through sugar fermentation. This alkali is ammonia produced from protein digests and possibly carbonates produced from fermentation of organic acid.

Five of the pathogens may be definitely differentiated by their ability to ferment certain of the carbohydrates.

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CRINKLE DISEASE OF STRAWBERRY¹

S. M. ZELLER AND E. K. VAUGHAN

The "crinkle" disease of strawberry was first noticed by C. E. Schuster and the senior writer in 1925.² Since that time the name crinkle has been applied to this disorder in California and Oregon.

In the Pacific Northwest the disease is wide-spread in the Marshall type of strawberry. Any quantity of stock of this variety entirely free from crinkle in western Oregon is rarely found. Other varieties as a rule are quite free from it, but they may in time show the symptoms when planted in close proximity to diseased Marshalls. Other varieties in which the symptoms have been noted are Corvallis, Dunlap, Gene, Sear's La Grange, Magoon, Missionary, Nick Ohmer, Norwood, O. S. C. No. 7, Howard 17 (Premier), Redheart (U. S. D. A. No. 632), U. S. D. A. Nos. 227-A, 400, 520, and 682. Occasional plants of the Ettersburg No. 121 and Clark Seedling affected with similar symptoms have been observed.

For the most part the effects of the disease have not been severe enough to arouse the concern of growers. Affected plants are not killed. They continue to yield, although the quantity of crop is doubtless not up to standard. The disease probably does not rank in destruction with root weevil, crown borer, or Rhizoctonia. Its general appearance and behavior are distinct from those of the other pests and diseases under like conditions.

Crinkle has the general appearance and behavior of a virous disease. It is perhaps more nearly like the yellows (xanthosis)³ than any other described strawberry disease.

In his reported occurrence of yellows in Oregon,⁴ Plakidas may have been mistaken. It now seems more probable that the disease observed and reported by him was, in fact, crinkle. Yellows is evidently quite restricted to California and in some scattered stocks originating from that State. It does not occur generally in Oregon, so far as the writers are aware, but has been seen in a few cases where it may have been introduced on planting stock from California. These cases have been cleaned up. Crinkle, on the other hand, is widely distributed in the Pacific Northwest and probably has spread into California through planting stock. Its origin, of course, is unknown.

¹ Published as Technical Paper No. 177 with the approval of the Director of the Oregon Agricultural Experiment Station. Contribution from the Department of Botany and Plant Pathology.

² Oregon Agr. Exp. Sta., Bienn. Rpt. 1924-1926: 94. 1926.

³ Plakidas, A. G. Strawberry xanthosis (yellows), a new insect-borne disease, Jour. Agr. Res. 35: 1057-1090. 1927.

⁴ Loc. cit., p. 1061.

Although no one person has studied crinkle throughout its known north and south range, it would seem from the observations of several workers that the symptoms are more evident in the growing season toward the southern limits of this range.

The two most characteristic symptoms are the crinkled condition and chlorotic character of the leaves. The former is undoubtedly due to the uneven distribution of the chlorotic areas in the early stages of leaf development. The rugose condition of the leaf surface seems to follow the chlorotic areas with no distinct pattern. (Fig. 1.)

These chlorotic areas at first are extremely localized, starting in very small, developing leaves as mere pin-point areas and expanding somewhat with leaf expansion. By transmitted light these areas show up plainly. Often the extremely chlorotic centers of these yellowed spots become necrotic, at first reddened and then brownish dead tissue resulting. It is not uncommon to find smaller leaves with many necrotic centers during the less favorable growing conditions from September to March. Besides this stippling, some leaflets exhibit most uneven chlorosis. As a rule, leaflets are yellower toward the margins. This yellowing may extend in streaks along a certain few veins toward the midrib. On the other hand, the veins, for the most part, may become "cleared." This may extend even into the finely netted venation. Together with the resultant shortening of veins, there may be more or less normal growth in the neighboring green tissues, producing various degrees of crinkled leaf surface.

With the uneven distribution of growth there results an uneven margin of the leaflets. The more or less regular dentation becomes a deeper crenation and an unnatural wavy lobing of the margins.

During any season of the year the whole of an affected plant is a lighter shade of green than normal. In the greenhouse, or in the field during late fall and winter plants lose most of their erect growth. The leaves produced under these conditions have short petioles, the whole plant presenting a flattened appearance. During favorable growing periods the affected plants may grow out of most of the symptoms and yet retain some characters to distinguish them from healthy plants. The leaves under these most favorable conditions do not have quite the uniform greenness and smooth surface exhibited by normal leaves, and there is a tendency for the leaves to arch downward or cup upward at the margins.

In late fall and winter the runner plants may show symptoms as characteristically as the parent plant. This is not always true in very fertile soils, as pointed out in more detail below. All affected varieties that have been observed with runner plants have shown the symptoms in the latter, and exceptionally well in runner plants of the Corvallis, Magoon, Marshall, Nick Ohmer, and Norwood.

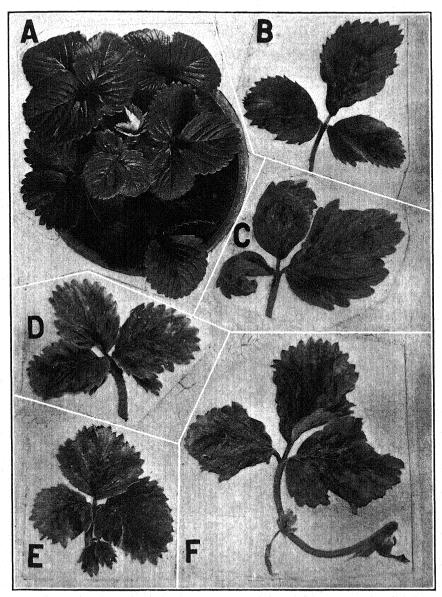


Fig. 1. A. Healthy Marshall strawberry plant in the greenhouse. B. Leaf from Missionary variety in the greenhouse. Note the pin-point and streak chlorotic areas due to crinkle disease. C. Leaf from Corvallis variety in the field, November. Note pin-point chlorotic areas, abnormal dentation, and curled leaflets due to crinkle disease. D-F. Leaves from Marshall variety in the field, November. D and E produced in late fall and showing characteristic symptoms. F produced in late summer and showing little chlorosis but characteristic crinkling and abnormal margins.

In the greenhouse, selections of runner plants have been made through two generations. Both healthy plants and those affected with crinkle were selected, segregated, and planted in pots, and the runner plants from these have been planted singly into pots of sterile soil. In all cases where parent plants were healthy the runner plants have remained healthy, but in cases where the parents were "crinkled" every runner plant from them has shown symptoms of crinkle sooner or later. This behavior has become the basis for selecting runner plants or for roguing fields to eliminate the disease from planting stock.

It was hinted above that in fertile soils runner plants do not always show symptoms soon after rooting. When affected runner plants were potted into fertile soil in the greenhouse sometimes the leaves produced for a time subsequent to potting were without symptoms, but in a few weeks the symptoms returned on leaves produced later. Whether this temporary recovery is entirely due to fertility or to some other factors, such as self-induced toxicity of the soil, is a question.

This temporary recovery is noticeable in some soils in the field and has a very practical bearing on selection of planting stock in the late fall. In plantings less than a year old one can not be sure to recognize the disease, while in older plantings the parents and most of the runner plants exhibit disease symptoms. This indicates that where autumn roguing is necessary planting the previous fall is desirable.

An investigation of the cause of crinkle is now under way at the Oregon Agricultural Experiment Station. In August of this year affected plants were sent to the U. S. Department of Agriculture, Division of Nematology, for examination, but no parasitic nematodes were consistently found in the leaves, buds, or roots. The symptoms and behavior of the disorder suggest the probability of a virosis rather than a disease of fungous or bacterial origin. Whatever the cause, there are indications that the disease spreads in the greenhouse and in the field.

Perhaps the best evidence of the transmission of the disease is the fact that some of the new hybrids distributed from the U. S. Department of Agriculture have contracted the disease in Oregon. These hybrids originated at Glenn Dale, Maryland, where George M. Darrow, of the U. S. Department of Agriculture, believes crinkle does not occur. Some of this stock was planted near the Marshall variety affected with crinkle at the Oregon Agricultural Experiment Station, Corvallis. Now, some of this stock growing at Corvallis and some of it, which has been distributed to growers elsewhere in the State, have developed crinkle.

In the greenhouse crinkle has shown up in the U. S. D. A. 227-A and the Missionary, both of which were growing in pots near affected plants of the Marshall variety. Whether this transfer took place in the greenhouse

or in the field before potting is not known, but these two varieties are not grown commercially here, being introduced for experimental purposes from Eastern or Southern States. In commercial fields the disease seems much more prevalent than a few years ago, sometimes more than half of the plants being affected by the third year.

Experiments have been started by the junior writer to determine the possible insect vectors and whether crinkle may be transmitted by means of grafting healthy and diseased tissues or by the injection of juices extracted from diseased tissues.

Fortunately, practical measures based on our present knowledge of the crinkle disease can be instituted to materially better the planting stock of affected varieties. The practicability of selection and roguing to eliminate crinkle from the Marshall variety has been demonstrated and has led to the certification of strawberry plants in the State of Washington.⁵ In the selection of plants and roguing for this certified stock the purpose was to eliminate all abnormal plants. Some of this stock, planted in several places in Oregon, shows almost complete elimination of crinkle.

Doubtless, where very high percentages of the disease exist, fields should be abandoned as sources of planting stock, but selection of normal plants could be advised in fields containing perhaps 10 to 20 per cent of crinkle, while roguing is practicable only in cases of less than about 5 per cent.

SUMMARY

In this paper is described for the first time a strawberry disease that, so far as known, is limited in distribution to the Pacific Coast States. Its symptoms and behavior in the field would suggest that it may be a virosis, although its transmission from diseased to healthy plants has not been proven experimentally. There is considerable circumstantial evidence that this disease, for which the name "strawberry crinkle" is proposed, is transmitted under field conditions. Practical methods of eliminating the disease from planting stock by means of roguing or selection are deemed successful.

⁵ Hunter, E. D. State certification of strawberry plants. Better Fruit 24 (9): 31-32. 1930.

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KEEPING QUALITY AS A FACTOR IN SELECTING NEW VARIETIES OF SMALL FRUITS

NEIL E. STEVENS

During the past 15 years much of my time has been given to the study of the rots of small fruits, especially those that develop after the fruit is picked. Most of the work directed toward the control of these rots has been done in active cooperation with the refrigeration and transportation projects of the Bureau of Plant Industry, with claims prevention officers of the transportation companies, and with sales companies and sales agents. This cooperation has been exceedlingly pleasant and profitable. There is, therefore, no danger of any misunderstanding of the statement that if I were to start another 15-year period on the same project I believe my cooperation would be chiefly with plant breeders.

I am conscious of the scientific and practical value of the information gained in the studies of refrigeration and transportation. Much progress has been made in the shipping and refrigeration of strawberries, for example, since that day in 1860 when C. S. Dod (1) shipped strawberries a distance of 474 miles by railroad in a refrigeration chest, in which berries were held in tin cups placed on a platform of wood resting on spiral mattress springs and on the top of the whole a box 4 in. deep holding ice, or even since 1868 when D. W. Davis (3, p. 444; 6, p. 574) of Detroit made an experimental shipment of strawberries from Cobden, Illinois, to Buffalo, New York, in a specially designed car. In this car, which was insulated, galvanized iron tanks to contain a mixture of ice and salt were arranged along the sides.

Strawberry growing in the South depends for its existence as a major industry on the possibility of delivering the fruit in salable condition to large northern markets, which means two things—a variety that will ship and adequate means of transportation. The same may be said of the other large fruit industries of the Southern States. W. A. Taylor (6) traces the successful development of the Georgia peach industry to the origination of a variety (the Elberta) better adapted to long shipments than any previously known and to the development of a car service adequate for fruit transportation.

The point I am trying to make is that of these two essentials, an adequate variety and proper transportation, too little attention has been given to the question of selecting a variety. In other words, this commercial structure we have erected, buttressed by careful handling, rapid transit, and refrigeration, rests on a rather weak foundation, for the fruit itself is of varieties that are of a poorer keeping quality than may well be obtained.

Moreover, whatever further improvements can be made in handling, refrigeration, and transportation may be even more advantageously applied to varieties of fruit having better carrying qualities than those now grown.

CHANGING CONDITIONS IN THE TRANSPORTATION OF PERISHABLE FRUIT

While it is, of course, possible that refrigeration equipment and methods may be greatly improved and perhaps radically modified in the future, it is evident that changing conditions in transportation of very perishable commodities in the eastern United States are rendering of relatively less value our present knowledge of refrigeration. The rapid increase in non-refrigeration truck transportation is hard to comprehend unless one is closely in touch with the development. A summary of motor-truck movement, prepared by the United States Department of Agriculture in cooperation with the Delaware State Board of Agriculture, points out:

"For strawberries, the shipments from the Eastern Shore section, (Maryland, Delaware and Virginia) by rail and boat in 1928 amounted to 2,131 carloads compared with a reported motor-truck movement equal to 2,396 carloads. It is interesting that records available indicate that, in 1926, the rail shipments of strawberries were 2,862 cars compared with 1,086 'carload equivalents' reported by motor-truck, thus showing a large increase in the use of the motor truck during the last two years."

"Motor truck shipments from the Eastern Shore in 1928 went to points as far distant as Boston and Buffalo."

A somewhat similar survey (4) of the region around Greater New York indicates that in 1928 almost ¾ (72.9 per cent) of the fruit and vegetable production of New York and New Jersey that moved into New York City was carried by truck.

The movement of strawberries from North Carolina to northern markets by motor truck began in a small way in 1926 or 1927. No exact figures are available, but it is known that in 1927 only about 20 loads of strawberries were delivered to Philadelphia² from North Carolina; all the rest came by rail. So rapidly did trucking of these commodities increase, however, that in 1929, 1,483 carloads were sent by rail as against 397 "carload equivalent" by truck. In 1930 the ratio was 756 cars by rail—467 "carload equivalents" by truck.

¹ Edwards, Brice, and J. W. Park. Motor-truck movement of fruits and vegetables from Delaware and the eastern shores of Maryland and Virginia—1928. 25 pp. U. S. Dept. Agr., Bur. Agr. Econ., in cooperation with Del. State Bd. Agr., Washington, D. C. 1929. (Mimeographed).

² Lensen, W. G., and J. W. Coleman. The Philadelphia strawberry market. Season of 1927. Summary. 8 pp. U. S. Dept. Agr., Bur. Agr. Econ., Fruit and Veg. Div., Market News Serv. 1927. (Mimeographed).

A future development, which is entirely possible with such expensive products as Florida strawberries and which has already been tried out, if newspaper reports may be credited, is airplane transportation.

The latest aid to refrigeration, solid carbon dioxide, appears, according to Brooks (2), less promising as a means of controlling rots of strawberries in transportation than of such fruits as dewberries, grapes, plums, and cherries.

THE POSSIBILITY OF SECURING VARIETIES OF BETTER CARRYING QUALITY

It is not necessary for present purposes to argue as to whether truck transportation has come to stay, whether solid carbon dioxide can be economically used, or whether strawberries should be carried in airplanes; the point I am trying to make is that small-fruit transportation is undergoing changes and that one of the present needs is to give attention to the selection of varieties on the basis of keeping quality.

To assist in this work it is certainly not necessary for the pathologist to become a plant breeder. Our friends, the geneticists, will welcome cooperation, and they assure me that selection among the results of their crosses is much more difficult than the crossing and propagation itself. The very least that the pathologists ought to do is to discover for each variety of fruit what characteristics seem to result in or at least are to be associated with better keeping quality. Armed with this information the breeder can work much more efficiently.

One thing must be evident—this plan certainly does not mean sacrificing eating quality for keeping quality. In making selection on the basis of keeping quality we shall concern ourselves only with varieties that have already been carefully selected for their horticultural characters, size, color, and flavor. As an illustration of the range of variation in keeping quality among varieties already selected for further study because of excellent horticultural characteristics and eating quality, two examples may be cited—the blueberries bred by F. V. Coville and the strawberries bred by G. M. Darrow, both of the Bureau of Plant Industry. In fact, it was my incidental observations of the great range in keeping quality shown by the plants grown in their trial plantings that first aroused my interest in this subject.

The difference in keeping quality of blueberry varieties of proved horticultural value and high eating quality and the tests by which these differences are determined experimentally were described several years ago in this journal (5). This work has already entered into commercial practice among blueberry propagators. The results of a number of such tests made each year on promising new blueberry crosses are placed in the hands of the growers and are considered by them in deciding what varieties to propagate.

A somewhat similar difference in keeping quality appears among strawberry varieties. (See Table 1).

TABLE 1.—Comparative keeping quality of fruit of 6 strawberry crosses and of the Missionary variety grown at Glenn Dale, Maryland, in 1929, as indicated by average percentage of sound berries at end of keeping test

Variety	Number of tests	Average percentage sound after 2 days at 20° C.	Average percentage sound after 1 week at 7-10° C.
632	4	95	78
542	4	91	91
Missionary	6	80	66
682	4	65	49
520	4	63	49
25	5	43	30
652	5	38	12

This table shows the wide range in keeping quality of the various crosses from approximately 91 per cent to only 12 per cent sound after a week in cold storage. It also shows that under growing conditions obtaining at Glenn Dale, Maryland, in 1929, two of the crosses, both of excellent eating quality, showed a keeping quality superior to that of Missionary, a standard variety used as a check.

The importance of breeding to secure small fruits suitable for canning and for frozen packs is already recognized (7). The suggestion that we test our small fruit crosses and select with a view to securing fruit of better quality or even that in the case of raspberries or strawberries we breed purposely for keeping quality is merely proposing that we do quickly and purposefully what is apparently done slowly and expensively (and more or less unconsciously) by a process of natural selection. It is obvious to any but the most casual observer that keeping quality must have been a large factor in the commercial survival of such important varieties as the Elberta peach, Klondyke strawberry, and Howes cranberry.

BUREAU OF PLANT INDUSTRY, WASHINGTON, D. C.

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BOOK REVIEW

Fischer, E., und E. Gäumann. Biologie der pflanzenbewohnenden parasitischen Pilze. 428 pp. Gustav Fischer, Jena. 1929.

Here at last is a book on principles of plant pathology and, better still, Several excellent books on plant diseases have appeared in the last few years, but most of them deal with the applied phases of plant pathology, with diseases arranged according to their causes or the crop Klebahn's "Grundzüge der allgemeinen Pflanzenplants they attack. pathologie" summarized some basic viewpoints but is now somewhat out of date and does not contain sufficient detail. It marked, however, a step in the right direction. But "Die Biologie der pflanzenbewohnenden Pilze" marks an epoch—the clear recognition that modern plant pathology is in part a pure science, not merely an applied one. The authors discuss basic principles and classify pathologic phenomena; they do not catalogue individual diseases. While they discuss only the pathogenic fungi and diseases caused by them, their book is one of the most notable contributions to the literature of plant pathology because it is a departure from the traditional viewpoint of diseases as a series of separate phenomena.

In a short introduction the authors discuss parasitism, saprophytism, and symbiosis. The main part of the book is divided into two parts: Part I deals with the prerequisites for the development of parasitic relations between pathogens and host plants, and Part II deals with the development of the parasitic relationships.

In Part I (The Prerequisites for the Parasitic Relation) is described quite fully what is known at present about natural susceptibility and resistance and changes in resistance caused by soil and meteorological factors, injuries, and the attack of other parasites. There is a brief discussion also of artificial immunization. This is followed by a section on the nature of resistance. The authors then consider the prerequisites on the part of the parasite for infection, including a good discussion of the natural differences in pathogenicity of parasitic fungi, variability in pathogenicity, and the influence of physiologic specialization. This is then followed by a description of the ways in which pathogens survive unfavorable conditions, the different kinds of inoculum, the methods of dissemination, and the factors affecting germination and growth.

In Part II (The Course of the Parasitic Relation) is given a summary of the various methods by which parasites cause infection, including the methods of entrance into host plants, the histological relationships between pathogens and hosts, the fate of the mycelia inside of the hosts, including effect of environmental factors on the development of diseases, and the fructification of pathogens on their host plants. This is followed by a consideration of anatomical and physiological changes caused by pathogens.

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The general sequence in the book is logical, although some pathologists might prefer to have the order reversed: to consider pathogens first and then the host plants. The principal topics might then be in the following order: the nature of pathogens, production of inoculum, dissemination, the phenomena of inoculation, incubation, infection, the environmental factors affecting the development of the diseases, and, finally, the effects of the disease on the host plants. There are, however, advantages and disadvantages in both arrangements, depending on personal preference.

The book is, of course, in German, but the style is clear and concise; there are numerous headings, which enable the reader easily to retain his orientation; copious literature citations are given at the end of each chapter; there are numerous tables and graphs; some good illustrations, although more could have been used to advantage; and the book, in general, is well made.

The most important fact is that the authors have organized between the covers of their book the most important facts concerned with the development of diseases caused by fungi. They have culled the literature, evaluated the contributions, and organized them into a logical system. This is so significant a contribution that criticism of the relatively few faults in presentation and interpretation, including a few minor inaccuracies, would seem mere fault-finding.—E. C. STAKMAN, University Farm, St. Paul, Minnesota, and C. R. ORTON, University of West Virginia, Morgantown, West Virginia.

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PATHOGENICITY OF THREE RED-CEDAR RUSTS THAT OCCUR ON APPLE¹

PAUL R. MILLER² (Accepted for publication February 15, 1932)

INTRODUCTION

An unusual and severe epiphytotic of quince rust on apples occurred in southern Indiana in 1929 and certain varieties of apples, such as Delicious and Winesap, which had heretofore been considered rust resistant, showed abundant calyx-end infection on the fruit.

There are 3 species of the genus Gymnosporangium infecting apples in Indiana. For clarity and convenience the 3 rust diseases will be referred to as apple rust, caused by Gymnosporangium juniperi-virginianae Schw., quince rust, caused by G. germinale (Schw.) Kern, and hawthorn rust caused by G. globosum Farl. Following are some aids in identifying the 3 fungi:

The color of the aeciospore wall of Gymnosporangium germinale is pale yellow as contrasted with the light chestnut brown wall of G. globosum and G. juniperi-virginianae. The peridium of G. germinale dehisces by irregular shredding and does not become revolute. The peridial cells of G. juniperi-virginianae become much curved when wet, with the inner and side walls sparsely rugose and the latter with ridges extending halfway across, as contrasted with the smooth inner and side walls of the peridial cells of G. globosum. The teliospores of G. germinale have carotiform pedicels ranging from 9 to $12\,\mu$ in diameter near the spore, as contrasted with the cylindrical pedicels of the other 2 species, $3-5\,\mu$ in diameter. The cells of the teliospore of G. germinale have only one germ pore per cell, which is

¹ Contribution from the Botany Department, Purdue University Agricultural Experiment Station, LaFayette, Indiana. Thesis presented to the Graduate School of Purdue University in partial fulfillment of the requirements for the M.S. degree.

² Junior Pathologist, Bureau of Plant Industry, U. S. Department of Agriculture. Grateful acknowledgment is made to Dr. E. B. Mains for valuable suggestions made during the earlier part of these investigations; to Dr. M. W. Gardner for helpful criticism during the latter part of the work and during the preparation of the manuscript; to Monroe McCown for numerous apple-rust collections; to Professors H. E. Thomas and J. A. McClintock for inoculation material; and to Dr. K. D. Doak for help and suggestions in photography.

located near the apex in the upper cell and near the pedicel in the lower cell, while in the other two species there are two germ pores in each cell located near the septum.

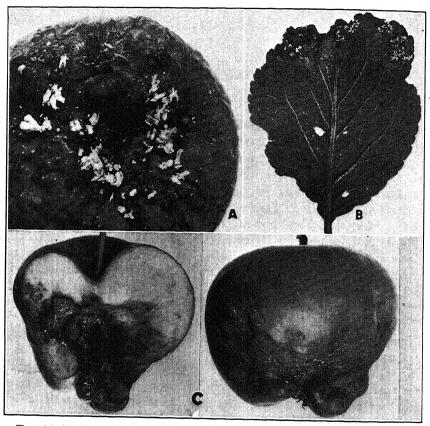


Fig. 1. A. Chimney-like aecia of Gymnosporangium germinale on calyx end of Winesap apple that had been stored in a refrigerator (enlarged). B. Leaf of Rome apple, torn across the tip before inoculation. Infection took place only along the torn edge. This indicates that the natural resistance that is acquired at maturity in susceptible varieties can be broken down by wounding. C. Fruit of Delicious variety of apples, showing stunting and malformation caused by calyx-end infection with quince rust (G. germinale) and the internal necrosis and cavity formation. Natural infection, 1929.

Quince rust was very destructive on apple fruit in 1929. In the lesions that are typical for Delicious, the tissue is killed while the fruits are small, and, due to the unequal growth, a very dwarfed and misshapen fruit results (Fig. 1, C). The internal tissues are killed to a considerable depth. It is not unusual for these fruits to be cracked open, exposing large cavities in

the flesh. For Winesap the surface of the lesion is usually of a water-soaked dark green color, with larger areas of the fruit surface involved. Aecia are not usually produced. However, occasionally aecia were produced on infected fruits that were stored in a refrigerator (Fig. 1, A).

The symptoms produced by hawthorn rust on apple leaves are very similar to those caused by apple rust except that the lesions are smaller, the aecia are fewer and grouped in the center of the lesion, and the peridial tubes are persistent, are longer than those of apple rust, and dehisce irregularly, never becoming revolute.

On the red cedar the 3 rusts can be differentiated by the following characteristics: At maturity the hawthorn rust gall is reddish in appearance, as contrasted with the greenish-brown apple-rust gall, and instead of pit-like depressions there are on this gall wedge-shape elevated areas, fewer and larger. From each of them a brown tongue-shape gelatinous horn emerges (Fig. 2, C), as contrasted with the slender cylindrical spore horns

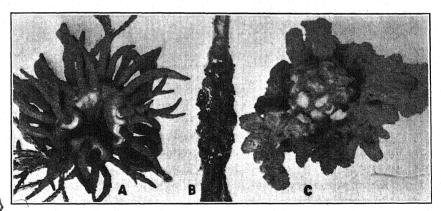


Fig. 2. Gymnosporangium galls on red cedar as they appear with teliospore sori gelatinized and expanded. A. G. juniperi-virginianae, showing the cylindrical spore horns. B. G. germinale, showing the broad wart-like spore masses. C. G. globosum, showing the large tongue-shape spore masses.

of the apple rust (Fig. 2, A). Quince rust produces inconspicuous cylindrical or spindle-shape, roughened swellings on the twigs and limbs of red cedar, which vary from 2 to 60 cm. in length and usually encircle the limb (Fig. 2, B).

VARIETAL SUSCEPTIBILITY IN INDIANA APPLE ORCHARDS

Apple varieties, such as Delicious, Winesap, and Stayman, had been considered rust resistant previous to 1929 in Indiana. In order to determine the resistance or susceptibility of varieties of apples commonly grown

in Indiana, field observations were made from August 15 to 21, 1929, in 18 commercial orchards in southern Indiana. Counts of at least 50 fruits and 100 leaves of each variety were made in each orchard. The records on fruit infection are shown in table 1 and on leaf infection in table 2.

TABLE 1.—Occurrence of apple rust (Gymnosporangium juniperi-virginianae) and quince rust (G. germinale) on fruit of apple varieties in Indiana orchards, 1929

	No. of fruits	Percentage of fruits infected				
Variety	examined	Apple rust	Quince rust			
Jonathan	100	20	0			
Rome	200	35	0			
Wealthy	150	25	0			
Winter Banana	250	10	1			
Ben Davis	150	12	0			
Winesap	250	0	8			
Baldwin	150	0	2			
Stayman	150	0	5			
Delicious	150	0	15			
King David	250	0	2			

It will be noted in table 1 that quince rust occurred on fruit of the varieties of Delicious, King David, Winter Banana, Winesap, Baldwin, and Stayman, none of which except Winter Banana were infected with apple rust. A few fruits of Winesap and Delicious were found bearing aecia, while the other varieties bore only pycnia.

Apple rust was severe on the Jonathan, Rome, Wealthy, Winter Banana, and Ben Davis varieties. Some Rome and Wealthy fruits bore aecia, the others only pycnia.

No hawthorn rust was found on the fruit.

As table 2 shows, the varieties Jonathan, Rome, Wealthy, Winter Banana, and Ben Davis were severely infected with apple rust. The lesions were large in most cases, and there were only a few on each leaf. Accia were produced in abundance on Rome and Wealthy, sparingly on Jonathan, and on Winter Banana and Ben Davis only necrotic flecks with pycnia were produced.

Hawthorn rust was found on the varieties Rome, Jonathan, Baldwin, Winesap, Northwestern Greening, and McIntosh. The lesions were smaller than those caused by apple rust. A few aecia were formed on Rome and McIntosh and only pycnia on the other varieties.

No leaf infection caused by Gymnosporangium germinale (quince rust) was found.

TABLE 2.—Occurrence of apple rust (Gymnosporangium juniperi-virginianae) and hawthorn rust (G. globosum) on leaves of apple varieties in Indiana orchards, 1929

Vonietre	No. of leaves	Percentage of leaves infected				
Variety	examined	Apple rust	Hawthorn rust			
Rome	500	40a	12b			
Ben Davis	200	22c	0			
Jonathan	300	25b	5d			
Wealthy	500	65a	0			
Winter Banana	100	10c	10 n			
Baldwin	100	0	8d			
Winesap	200	0	4 d			
Northwestern Greening	100	0	94			
McIntosh	100	0	1 b			

a Aecia abundant.

TELIOSPORE AND BASIDIOSPORE GERMINATION

Red-cedar branches bearing galls caused by Gymnosporangium juniperivirginianae were brought into the greenhouse January 5, 1930, and the cut ends put in water. At the end of 2 weeks these galls showed signs of pushing out spore horns. On January 23 some of these branches were put in a damp chamber and on January 31 spore horns had been pushed out. Portions of these spore horns were put in drops of water on slides and the slides were then placed in a moist chamber. Normal germination of the teliospores occurred.

During the process of spore-horn protrusion some of the leaves on the twigs bearing galls turned yellow and dropped off. Since this yellowing was thought to be due to the excessive drain by the rust on the food supply of the host, sucrose was added to the water, and this seemed to cause the infected cedar twigs to maintain their viability longer. It was necessary to make a fresh cut across the basal end of the limb every few days and to change the solution frequently because of bacterial growth. With this procedure it was possible to obtain an abundance of teliospores for germination and inoculation tests over a period of several months.

Reed and Crabill (13) found 15° C. to be the optimum temperature for the germination of the teliospores of *Gymnosporangium juniperi-virginiànae*, while Weimer (15) found it to be 22–25° C.

In order to determine the most favorable temperature conditions for germination of teliospores and basidiospores of the 3 species of rust under

b Aecia present but not abundant.

c Necrotic flecks with pycnia.

d Pycnia only.

consideration, germination tests in water were made at temperatures ranging from 0 to 36° C. Approximately 5,000 of each of the 2 spore forms of the 3 species of rust were counted. The results are given in table 3. The average percentage germination is given, but with teliospores this can be no more than a general average because there is such a variation in the germinability of these spores, depending upon their maturity, location on the spore horn, and some undetermined factors. The results show that the teliospores of the 3 species of rust will germinate well between 20 and 28° C., with the optimum at about 24° C., while the basidiospores germinated best at 16° C, and fairly well between 12 and 24° C. Three hours at the optimum temperature were necessary for basidiospores to be produced.

TABLE 3.—Effect of temperature on the germination of teliospores and basidiospores of the three apple rust fungi

Tem-	Gymnosporangium juniperi-virginianae		G. glo	bosum	G. germinale			
perature used	Teliospore germina- tion	germina-		germina- tion germina- germina- tion germina- tion		Basidio- spore germina- tion	Teliospore germina- tion	Basidio- spore germina- tion
$^{\circ}C.$	Per ct.	Per ct.	Per ct.	Per ct.	Per ct.	Per ct.		
0	0	0	0	0 0	0	0		
4	.05	0	.5	0 2	1	0		
8	2	.01	1	.4	1.5	.2		
12	5	30	3	27	2	8		
16	8	65	6	50	4	45		
20	52	50	41	41	37	30		
24	76	17	60	12	50	16		
28	50	1	34	.07	28	.6		
32	.2	0	.5	0	.7	0		
36	0	0	0	0	0	0		

In each of the 3 rust species, an abnormal type of teliospore germination occurred at high temperatures, in which long promycelia were formed but no basidiospores were developed. The basidiospores normally germinate by one or more germ tubes, but, under certain conditions, instead of a germ tube being produced, a sterigma similar to that formed on the promycelium was put forth and on the end of this a secondary basidiospore was formed. The secondary basidiospores appeared to be similar to the primary ones except that they were smaller. Production of such basidiospores could be brought about at will by high temperature and abundant water.

INOCULATIONS OF APPLE VARIETIES AND OTHER AECIAL HOST SPECIES

In May, 1930, inoculations were made on 10 commercial varieties of apples in the orchard, apple seedlings, and potted plants of quince, pear, Sorbus sp., and Aronia. Approximately 50 leaves and 25 fruits of each apple variety were inoculated with each of the 3 species of rust. The method used was to suspend a portion of the gelatinized spore horn on a wire about 3 in. above the tissue to be inoculated. The apple leaves were pulled between the fingers and rubbed gently to remove the bloom, so that the water would adhere to the leaf. The leaves or fruits were atomized with water and a piece of cotton soaked in water was wrapped around the stem to insure high humidity in the glassine bag that was put over the spore mass and parts to be inoculated. The glassine bags were closed and fastened with metal clips and were left on about 24 hours.

The results presented in table 4 show that infection with apple rust (Gymnosporangium juniperi-virginianae) was secured on the fruits of Rome, Wealthy, Ben Davis, and Grimes and the leaves of Rome, Wealthy,

TABLE 4.—Inoculations in the orchard with apple rust (Gymnosporangium juniperivirginianae)

77	Fr	uit	Leaves			
Variety or species inoculated	No. inoculateda	No. infected	No. inoculateda	No. infected		
Rome	15	12	17	13		
Wealthy	10	6	17	17		
Ben Davis	9	5	7	2		
drimes	12	2	15	12b		
King David	27	0	24	15b		
Jonathan	20	0	16	10		
Maiden Blush	12	0	30	0		
Delicious	18	0	26	0		
Stayman	26	0	23	0		
Winesap	26	0	28	0		
Pear			10	0		
Sorbus sp.c			9	0		
Aroniae			12	0		

a Number inoculated that did not drop prematurely.

Ben Davis, Grimes, King David, and Jonathan. The symptoms on these infected fruits and leaves were identical in appearance with those occurring in nature. Pycnia appeared about 3 weeks after inoculation. Acciospores produced on the inoculated fruit and leaves were examined and found to

b Flecking with no pycnia produced.

c Potted plants in greenhouse.

agree in shape and measurement with the aeciospores of this species of rust. On inoculated Grimes and King David leaves, only necrotic spots were produced and these rarely reached a diameter of one mm. No pycnia were produced in these spots. Inoculated leaves of pear, *Sorbus* sp., and Aronia failed to become infected.

A greater percentage of the inoculated fruit and inoculated leaves dropped prematurely from the varieties that were susceptible than from those that were resistant.

The results presented in table 5 show that no fruit infection was obtained with hawthorn rust (Gymnosporangium globosum). Leaf infection with

TABLE 5.—Inoculations in the orchard with hawthorn rust (Gymnosporangium globosum)

	Fr	uit	Leaves		
Variety or species inoculated	No. inoculateda	No. infected	No. inoculateda	No. infected	
Maiden Blush	15	0	30	22b	
Grimes	18	0	27	20b	
Ben Davis	14	0	15	12d	
Jonathan	17	0	13	9d	
Rome	25	0	12	8e	
Wealthy	16	0	11	6e	
Sorbus sp.c			16	16f	
Aroniac			35	20b	
Pearc	8	0	15	12b	
Stayman	14	0	26	0	
Winesap	12	0	24	0	
Delicious	30	0	26	0	
King David	18	0	21	0	

a Number inoculated that did not fall prematurely.

hawthorn rust was obtained on the following apple varieties: Maiden Blush, Grimes, Ben Davis, Jonathan, Rome, and Wealthy, and on the leaves of Sorbus, Aronia, and pear. The symptoms on these infected leaves were identical with those occurring in nature. Spore and peridial tube measurement agreed with the measurements given for *G. globosum*. On the leaves of Maiden Blush and Grimes apples, Aronia, and pear, only necrotic spots without pycnia were produced. On Ben Davis and Jonathan only pycnia

b Necrotic flecks with no pycnia produced.

c Potted plants in greenhouse.

d Only pycnia produced.

e A few aecia produced.

f Aecia abundant.

were produced, on Rome and Wealthy a few aecia were produced, and on *Sorbus* sp. there were abundant aecia.

The results of the inoculations with quince rust (Gymnosporangium germinale) are given in table 6. Infection with quince rust was secured on

TABLE 6.—Inoculations in the orchard with quince rust (Gymnosporangium germinale)

Variety or energies	Fr	uit	Leaves			
Variety or species inoculated	No. inoculateda	No. infected	No. inoculated ²	No. infected		
Delicious	20	13	16			
Winesap	17	9	13	0		
Stayman	8	6	17	0		
Wealthy	12	5	22	0		
King David	14	0	26	0		
Maiden Blush	20	0	24	0		
Ben Davis	16	0	15	0		
Jonathan	11	0	21	0		
Grimes	15	0	35	0		
Rome	14	0	20	0		
Quinceb	0	0	15	15		

a Number that did not drop prematurely.

the fruits of 4 varieties: Delicious, Winesap, Stayman, and Wealthy. The symptoms were identical with those occurring in commercial orchards on the same varieties in 1929. No visible infection on the apple leaves resulted, but quince leaves were infected. The lesions were confined to the veins, which were swollen and necrotic at the point of infection.

Teliospore material of quince rust obtained from Prof. H. E. Thomas, Ithaca, New York, J. A. McClintock, Knoxville, Tennessee, and from La Fayette, Indiana, in 1931, was used in inoculation tests in the orchard for the purpose of determining whether or not physiologic forms exist in these different localities. Six varieties of apples were used as differential hosts: Delicious and Winesap, since these had previously been found to be very susceptible under Indiana conditions; Stayman and Wealthy (moderately susceptible); and Jonathan and Rome (resistant). The methods of inoculation used were similar to those used in 1930.

The results as indicated in table 7 show that the varieties Delicious, Winesap, Stayman, and Wealthy were susceptible to the inoculum coming from the 3 localities. Jonathan and Rome were resistant, with the exception that 2 Rome fruits inoculated with the spores from Tennessee developed symptoms that were typical for an early stage of quince rust. The fruits

b Potted plant in greenhouse.

were observed at weekly intervals from the first sign of infection until they were mature, with no difference of symptoms being detected. As in 1930, no visible leaf infection occurred.

TABLE 7.—Inoculation of apple fruits with Gymnosporangium germinals from New York, Tennessee, and Indiana, 1931

	New	York	Tenn	essee	Indiana		
Variety	No. fruits inoculated	210122		No. infected	No. fruits inoculated	No. infected	
Delicious	25	17	25	15	25	19	
Winesap	10	9	10	10	10	8	
Stayman	25	14	25	7	25	15	
Wealthy	10	6	10	8	10	5	
Jonathan	25	0	25	0	25	0	
Rome	10	0	10	2a	10	0	

^a Two fruits exhibited a dark green, puckered appearance.

EXCISED APPLE LEAVES CARRIED ON SUCROSE SOLUTION

In order to extend the period of time during which inoculation work could be carried out, a modification of the methods used by Mains (12), Clinton and McCormick (1), and Giddings and Leonian (10) has been employed. Excised apple leaves were carried on a 6 per cent sucrose solution in Petri dishes for 2 months, or long enough after inoculation for pycnia and aecia to be produced.

In the earlier experiments a galvanized 4-in. mesh wire was put in the Petri dish, and the petiole of the leaf was inserted through this, so that it was the only part of the leaf in contact with the solution. Later the leaf petioles were inserted through holes in thin layers of cork that were floated in the nutrient solution. The leaves were first washed in running water. The nutrient solution was changed every 5 days, and dead portions of the leaves that were noticed were cut off and removed. The leaves were inoculated in the dishes by atomizing with a basidiospore suspension.

It was found early in this work that the time of day that the leaves were removed from the tree was of great importance. Leaves removed from the tree in the late afternoon remained viable much longer on the sucrose solution than those taken in the morning. This may be accounted for by the probability that leaves taken in the late afternoon contain much starch, while the leaves taken in the morning are low in starch.

With this method of keeping excised apple leaves alive on a sucrose solution, studies were made of the relation of wounds to leaf infection and of heterothallism, as will be presented in the 3 following sections.

RELATION OF WOUNDS TO SUSCEPTIBILITY OF APPLE LEAVES TO APPLE RUST (GYMNOSPORANGIUM JUNIPERI-VIRGINIANAE)

Fulton (8) noted that apple leaves become resistant to rust as they mature. Giddings and Berg (9) observed that in nature mature apple leaves may become infected through insect injuries.

An apple leaf was accidentally torn with a metal clip in the process of bagging an inoculated twig and infection occurred only along this wounded portion. Following this discovery, mature apple leaves of 5 varieties, some of which are considered rust resistant and others of which are susceptible, were used in inoculation experiments with apple rust. Inoculations were made on Rome, Jonathan, Wealthy, and Winesap leaves under field conditions, using the same methods as above, and also on excised Rome, Jonathan, Wealthy, and Delicious leaves carried on sucrose solution in Petri dishes. The leaves were wounded by tearing them slightly, previous to inoculation. Uninjured leaves were inoculated at the same time.

The results as given in table 8 show that no unwounded leaves were infected and that wounded leaves of the susceptible varieties Rome, Jonathan, and Wealthy became infected, while those of the resistant varieties Winesap and Delicious did not. Infection took place only along the wounded portion of each leaf (Fig. 1, B). These results may be interpreted to indicate that the resistance that is shown by mature leaves of susceptible varieties has a morphological basis.

Leaves of the varieties Winesap and Delicious, which are naturally resistant when young, did not become infected when wounded and inoculated, as did those of susceptible varieties, which had acquired resistance because of their maturity.

TABLE 8.—Relation of wounds to infection of apple leaves with apple rust (Gymnosporangium juniperi-virginianae)

		Leaves not	wounded	Leaves wounded		
Location	Variety	No. inoculated	No. infected	No. inoculated	No. infected	
	Rome	20	0	20	12	
0-4	Jonathan	32	0	32	15	
On tree	Wealthy	16	0	16	13	
	Winesap	12	0	16	0	
	Rome	6	0	6	4	
	Jonathan	6	0	6	2	
In Petri dish	Wealthy	6	0	6	5	
	Delicious	6	0	6	0	

INDICATIONS OF HETEROTHALLISM IN APPLE RUST (GYMNOSPORANGIUM JUNIPERI-VIRGINIANAE)

With methods based on Craigie's work (2, 3, and 4) on heterothallism in *Puccinia graminis*, an attempt was made to determine whether or not apple rust (*G. juniperi-virginianae*) is heterothallic. Tests were carried out under 3 conditions in 1930, viz, potted seedlings growing in the greenhouse, orchard or field conditions, and detached leaves carried in Petri dishes on a sucrose solution.

In the orchard 60 leaves, each bearing only a single pyenial rust lesion, were found and tagged. At the proper time, which was about June 1, the pyenial exudate of 22 of these single lesions was mixed so that each lesion received exudate from several others. Twenty of these lesions had produced aecial sori containing aeciospores about July 20 (Table 9). On the 16 leaves bearing single pyenial lesions on which the pyenial exudate was not mixed only 9 produced aecia. Even though these leaves were not bagged to protect them from insects, it may be significant that 90 per cent produced aecia where the pyenia exudate was mixed and only 56 per cent where it was not mixed (Table 9).

A very dilute spore suspension of basidiospores was atomized on the leaves of potted seedlings, May 15. On certain leaves only a single lesion resulted. These leaves bearing single lesions were bagged separately, and at the proper time, which was about June 8, the pyenial exudate on 12 of those isolated pustules was mixed. All of these lesions had produced aecia bearing aeciospores by July 30 (Table 9). On 7 isolated lesions the exudate was not mixed and no aecia were formed.

A basidiospore suspension was atomized on numerous detached leaves carried on sucrose solution in Petri dishes. On 13 leaves, only a single lesion per leaf resulted. On 8 of these leaves the pycnial exudate was mixed and 6 of these produced aecia (Table 9). Of the 5 leaves on which the pycnial exudate was not mixed only one produced aecia. This one exception may be explained by the possibility that it was a compound lesion derived from two or more basidiospores.

A few monosporidial lesions were obtained by the following methods: Drops of a very dilute spore suspension were put on slides and examined under a microscope. When a drop was found containing less than 5 basidiospores, an attempt was made to withdraw one spore by the aid of a bulb pipette attached to the mechanical stage. The spores remaining in the drop were counted and, if one less spore remained, it was assumed that one spore was in the pipette. The contents of the pipette were released on a leaf and by this method of inoculation a lesion was obtained on each of 8 leaves floated on sucrose solution. These were considered as monosporidial lesions.

The pycnial exudate of 4 of these was mixed and each of these lesions produced aecia (Table 9), while no aecia were formed by the other 4 lesions, where the exudate was not touched.

TABLE 9.—Tests indicating heterothallism in Gymnosporangium juniperi-virginianae

	Lesions with pycnial exudate not mixed			Lesions with pycnial exudate mixed			
	Total number	Number with aecia	Percent- age with aecia	Total number	Number with aecia	Percent- age with aecia	
Orchard	16 7 5 4	9 0 1 ^a 0	56 0 20 0	22 12 8 4	20 11 6 4	90 92 75 100	

^a Possibly a compound lesion.

Although the numbers of lesions in these tests are small, the indications are that *Gymnosporangium juniperi-virginianae* is heterothallic in the sense that the transfer of pycnial exudate may be necessary for accium production in monosporidial lesions.

INDICATIONS OF HETEROTHALLISM IN HAWTHORN RUST (GYMNOSPORANGIUM GLOBOSUM)

Fifty hawthorn leaves, each bearing an immature single pycnial lesion caused by Gymnosporangium globosum, were taken from the trees May 12, 1931, and floated on a 6 per cent sucrose solution. At the time of exudation of the pycnia, the exudate was transferred from one leaf to another on 25 leaves, each lesion receiving the exudate from several others. The pycnial exudate on the other 25 leaves was not touched. All leaves were protected from insects. It is interesting to note that the drops of exudate that had received foreign exudate dried down within 24 hours after the process, whereas the unmixed exudate remained for 30 days. By July 17, 1931, 17 of these leaves bearing the mixed exudate had produced aecia with mature spores. The remaining 8 leaves did not bear aecia. Of the 25 leaves bearing the unmixed exudate 4 had produced aecia, the remaining 21 did not.

Two hundred leaves similar to the above were taken from the trees June 4 and were treated in a manner similar to the above. Of the 100 leaves on which the exudate was mixed, 92 per cent had produced aecia by August 15, whereas only 3 per cent of the remaining 100 leaves bearing the unmixed exudate bore aecia.

Ninety leaves, each bearing a single pycnial lesion, were taken from the hawthorn trees June 10 and were put on the nutrient solution. Instead of promiscuous mixing of the exudate, the exudate from a single lesion was transferred to a series of 25 leaves, with special care to sterilize the needle between each transfer. Another series of 40 leaves received the exudate from another single lesion. By August 15, 1931, 22 of the above 25 leaves had produced aecia, and 36 of the 40 had done likewise. Although there is a possibility that the 2 individual lesions from which exudate was transferred were compound (the result of infection by more than a single basidiospore), this is not probable since none of the 25 leaves, each bearing a single lesion with the exudate undisturbed, used as checks, produced aecia. According to Craigie's work (5) on Puccinia graminis, one would have expected only about half of these lesions thus treated to produce aecia.

One hundred and fifty hawthorn leaves, each bearing a single pycnial lesion of *Gymnosporangium globosum*, were tagged on May 15, 1931. At the time of exudation of the pycniospores, the exudate was mixed on 75 of these leaves, each lesion receiving the exudate from several other lesions. The exudate of the remaining 75 leaves was not touched. All were unprotected from insects. By August 25, 70 of the leaves bearing the mixed exudate had produced aecia, whereas only 46 of the leaves bearing the unmixed exudate bore aecia.

FACTORS INFLUENCING AECIOSPORE GERMINATION

Heald (11), Reed and Crabill (13), Weimer (15), and Doran (6) experienced difficulty in obtaining good germination of aeciospores of *Gymnosporangium juniperi-virginianae*. Fukushi (7) and Thomas and Mills (14) have found that exposing aeciospores of apple rust to low temperatures increases their germinability.

Various tests were made to determine the factors influencing germination. Acciospores of Gymnosporangium juniperi-virginianae were germinated in drops of sucrose solution ranging from 0.2 to 6 per cent on glass slides in Petri dish moist chambers. The germination with the sugar solution was essentially the same as was obtained in the tap-water checks. Similar results were obtained with an extract of red-cedar leaves. Acciospores germinated in darkness equally as well as in daylight.

Temperature plays an important part in the germination of aeciospores. Counts of several thousand aeciospores of the 3 species of rust under consideration, germinated at temperatures ranging from 4 to 32° C. at intervals of 4°, have been made. The minimum, optimum, and maximum temperature at which these aeciospores germinate is fairly constant, but the percentage germinating is extremely variable, depending upon the time of the year these germination trials are made and the method of obtaining the

aeciospores from the sorus. If the aeciospores were dusted directly from the leaf onto the slide rather than scraped from the pustule, more uniform results were secured, presumably because only apical, mature spores are thus liberated.

No germination was secured at temperatures lower than 6° C. for Gymnosporangium juniperi-virginianae and G. globosum, while 9° C. was the minimum temperature for G. germinale. The optimum temperature for the germination of aeciospores of G. juniperi-virginianae and G. globosum was found to be 24° C., while the aeciospores of G. germinale germinated best at 16° C. Fairly good germination was secured from G. juniperi-virginianae and G. globosum from 16 to 28° C., while for G. germinale the range was 12 to 20° C. No germination was secured above 32° C. in any of the 3 species.

In August, 1929, apple leaves bearing aecia of Gymnosporangium juniperi-virginianae were placed in cheesecloth bags and suspended about 2 ft. from the ground from a limb of a tree, so as to be under natural environmental conditions. Germination tests of these aeciospores were made each month for a year (Table 10). All of these spores were germinated at 24° C., as this had previously been found to be the optimum temperature for aeciospores of G. juniperi-virginianae. The percentage of germination was determined by counting 500 aeciospores that were in proper conditions for germination. It is interesting to note that only a small percentage of the aeciospores germinated in August but that the germination percentage increased gradually throughout the fall and winter and reached its highest point in March at 84 per cent. Then, there was a more or less sudden drop, with a few aeciospores germinating even as late as the following August.

In August, 1930, a similar experiment was started and this time aeciospore-germination tests were made twice a month. The same tendency toward increased percentage of germination in the winter was noted (Table 10). On August 1, 4 per cent germination was secured; September 1, 6 per cent; October 1, 14 per cent; November 1, 20 per cent; December 1, 35 per cent; January 1, 46 per cent; February 1, 20 per cent; and March 1, no germination was secured. The cause for the decrease in germination of aeciospores in February and March, 1931, as contrasted with 1930, is not known. However, these months were unusually warm in 1931, and the leaves bearing the aeciospores were invaded by saprophytic organisms.

Tests of a similar nature were made on aeciospores produced in 1931, with results as shown in table 10. The germination percentage increased from 8 in August to 26 in January.

TABLE 10.—Longevity of aeciospores of Gymnosporangium juniperi-virginianae and the effect of overwintering on their germinability

1929 Aecio	spores	1930 Aecios	pores	1931 Aecios	spores
Dates of germination testsa	Percentage of germination	Dates of germination tests	Percentage of germination	Dates of germination tests	Percentage of germination
August, 1929 September October November January, 1930 February March April May June July August	12 12 32 25 50 50 72 84 44 13 7 1 A few weak germ tubes	August 1, 1930 '' 15	4 7 6 15 14 15 20 35 35 30 46 45 20 8	August 1, 1931 '' 30 October 30 November 15 '' 30 December 15 January 4, 1932	8 7 14 22 20 22 26

^a Approximately the 15th of each month.

Since the aeciospores germinated in March and April, at which time new growth is present on the red cedars, the possibility of spring infection of red cedars is suggested.

SUMMARY

The symptoms produced on the apple fruit by Gymnosporangium germinale are very different from those produced by G. juniperi-virginianae in that there is no yellowing on the surface, the fruit is misshapen due to the early killing of the tissue, and often there is considerable internal necrosis, rendering the fruit worthless.

The fruits of the following apple varieties were found infected with apple rust (Gymnosporangium juniperi-virginianae) in southern Indiana in 1929: Jonathan, Rome, Wealthy, Winter Banana, and Ben Davis. The leaves of Rome, Ben Davis, Jonathan, Wealthy, and Winter Banana varieties were infected with apple rust. Quince rust (G. germinale) occurred on fruit of Winter Banana, Winesap, Baldwin, Stayman, Delicious, and King David. The leaves of Baldwin, Rome, Jonathan, Winesap, Northwestern Greening, and McIntosh were infected with hawthorn rust (G. globosum).

Successful inoculations were made in the orchard with Gymnosporangium juniperi-virginianae on the fruit and leaves of Rome, Ben Davis, Grimes, and Wealthy and on the leaves of Jonathan.

Successful inoculations were made with Gymnosporangium globosum on the foliage of Maiden Blush, Rome, Ben Davis, Jonathan, Grimes, and Wealthy and, in addition, on Aronia, pear, and Sorbus sp. Only necrotic flecks were produced on Maiden Blush, Grimes, Aronia, and pear. Aecia were produced on Rome, Wealthy, and Sorbus sp.

Successful inoculations were made with Gymnosporangium germinale on the fruit of Delicious, Winesap, Stayman, and Wealthy varieties, and on quince leaves. The fruit symptoms produced resembled those resulting from natural infection.

The leaves of varieties easily infected with Gymnosporangium juniperivirginianae in the young stage with production of pycnia and aecia are not naturally infected at later stages of maturity. When however, mature leaves of such varieties (Rome, Jonathan, Wealthy) were wounded, inoculation was successful and pycnia and aecia were produced. On wounded leaves of resistant varieties, such as Winesap and Delicious, inoculation was not successful.

For use in inoculation or for studies of heterothallism apple leaves removed from the tree at the end of the day were found to remain alive on a 6 per cent sucrose solution in Petri dishes for two months, which was sufficient time for aecia to develop.

In both Gymnosporangium juniperi-virginianae and G. globosum, the majority of lesions resulting from infection by single basidiospores do not produce aecia. When the pycnial exudate from such lesions was mixed, the majority of the lesions produced aecia. This indicates that these rusts are heterothallic.

The optimum temperature for teliospore germination for G. juniperi-virginianae, G. globosum, and G. germinale was found to be 24° C.; for the basidiospores of the 3 species 16° C.; the aeciospores of G. juniperi-virginianae and G. globosum germinated best at 24° C.; while the optimum temperature for G. germinale was found to be 16° C.

At high temperatures (32 to 36° C.) the three rust species exhibited an abnormal type of teliospore germination in which long promycelia with no basidiospores were produced. Secondary basidiospores were formed under conditions of high temperature and abundant water.

Aeciospores of Gymnosporangium juniperi-virginianae lived over winter and germinated well in March and April. Some aeciospores germinated one year after collection. Aeciospores scraped out did not germinate as well as those dusted out.

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THE PROPERTIES OF PLANT VIRUSES FROM DIFFERENT HOST SPECIES¹

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Not only are the properties of plant viruses of general interest in relation to their bearing on the nature of a virus and on the control of the diseases that they cause, but they may serve in whole or in part as a means of isolation, differentiation, and classification of certain groups of viruses. Previous investigations have, in general, shown that the properties of a particular plant virus, such as that of ordinary tobacco mosaic from tobacco, are fairly definite and constant characters. While some variation has been claimed, it has not ordinarily been beyond that of normal biological variation or such as may be explained either through differences in material and methods or through experimental error.

Very little reliable information appears to exist, however, relative to the influence of different host species on the properties of any particular virus. If the source of the inoculum markedly affected the properties of a virus, this would naturally have some bearing on the nature and control of a virus disease and would perhaps complicate the value of property studies for purposes of differentiation.

The problem of the influence of the host species on the properties of a virus appeared, therefore, to be sufficiently important to warrant our making a fairly detailed investigation, such as is presented in this paper, even though the evidence, as it accumulated, indicated that the virus properties were not influenced in any material way by the host species in which the virus was propagated and from which it was extracted.

EARLIER INVESTIGATIONS

The studies by Allard (1, 2) and others on the ordinary tobacco-mosaic virus and those by Doolittle (3) on the cucumber-mosaic virus on the respective host plants showed that these viruses possessed fairly definite properties. When these properties are compared, it is evident that they are distinctly different in most particulars, although the significance of this fact was not at first realized and their similarity rather than their difference was emphasized. During this period there was a tendency to assume that apparently different viruses diseases were due to essentially the same causal agent and to account for the differences in properties and behavior on the basis of the

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particular host concerned. This belief, which may be illustrated by Walker's (11) conclusion that it is possible to change the property of a virus radically by transferring it to another host, was quite generally accepted. McKinney (10), considering this question from a somewhat different angle, suggested that the rapid loss of the infectious property of expressed cucumber-mosaic virus may be due in part to the nature of the cucumber-plant fluids and that the thermal death point of the tobacco-mosaic virus depends on the nature of the plant extract. More recently, on the other hand, specific plant viruses have been reported in increasing numbers with little or no evidence supporting the suggestion that a virus is a labile entity.

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MATERIALS AND METHODS

The method of experimentation used in this investigation consisted simply in inoculating the specific viruses used to a variety of susceptible host species. When infection on these plants was evident, extracts were made and treated as desired, following which inoculation was made to a series of tobacco plants to determine the effect of the treatment on the virus. A considerable number of minor difficulties may naturally arise in performing trials of this sort, but, in general, these will be passed over as having no significant bearing on the results or conclusions.

Four viruses were selected for the study, namely, the ordinary tobaccomosaic virus (tobacco virus 1 (7)), the cucumber-mosaic virus (cucumber virus 1 (7)), the tobacco-ring-spot virus (4), kindly furnished by Dr. S. A. Wingard, and the "spot-necrosis virus," now recognized as a combination of two viruses (9). Particular attention was paid, however, to the "mottle" virus constituent of the combination.

The host plants used as sources of inoculum were primarily of the family Solanaceae, chiefly for the reason that these were for the most part susceptible to all the viruses used. In a few cases representatives of other plant families were used, i.e., cucumber and Martynia. In general, however, the range in type of hosts used is believed to be sufficient for the purposes of the investigation. Tobacco, Nicotiana tabacum L., was always used more or less as a standard or control. Two other species of this genus were also frequently used, namely, N. rustica L. and N. glutinosa L. Other host species commonly used were tomato, Lycopersicon esculentum Mill; pepper, Capsicum annuum L.; ground cherry, Physalis pubescens L.; petunia, Petunia violacea Lindl.; and eggplant, Solanum melongena L. Nicandra physaloides (L.) Pers., Solanum atropurpureum Schr., Datura stramonium L., Cucumis sativus L., Apium graveolens L., Martynia louisiana Mill., Solanum nigrum L., S. miniatum Bernh., S. tuberosum L., S. carolinense L., Spinacia oleracea L., and other species were less frequently included. Difficulties in securing infection or good symptoms with some of the viruses on

certain hosts account for the apparent omission of what might otherwise prove interesting comparisons. In other cases a lack of seed of particular hosts resulted in a failure to secure adequate repetition of trials. In general, however, it was planned to run at least 3 separate trials with each virus from each host, inoculating 5 tobacco plants for each test.

The method of inoculation used was at first the cotton-needle prick and scratch method. Later, the rubbing method of Holmes (6) was used, especially in the dilution trials, since a higher percentage of infection could be secured by this method at high dilutions. The trials are only comparative, however, and usually the only question involved was whether the virus extract was or was not infectious following a given treatment. The percentage of infection secured was not usually regarded as of particular interest in the present trials.

The properties of the virus may naturally include a considerable number of characteristics, but for the present studies only 4 properties were selected for investigation, namely, the thermal death point, longevity in vitro, tolerance to dilution, and resistance to certain chemicals.

The methods used in the determination of these properties have been as simple as possible, first because they were intended, in any case, only to be comparative, and secondly because any attempt at too close refinement in technique might serve not only to complicate the results but also to make the usefulness of the methods too laborious to be practical.

The thermal-death-point determinations were made by placing about 2 cc. of freshly extracted juice in thin, stoppered test tubes (care being taken not to drop any extract on the sides of the tube) and placing these in an agitated constant-temperature water bath for 10 minutes, after which the tubes were rapidly cooled in water. With certain methods of inoculation, such as the leaf-mutilation method on potato, it is impractical to work with much less than 2 cc. of inoculum. It has, therefore, seemed advisable not to work with too small quantities of material in determinations of this type.

The aging tests were made by placing the extracts in stoppered test tubes for the required lengths of time in a darkened drawer in a greenhouse where the temperature usually ranged between $80-90^{\circ}$ F. The dilution tests were made in the ordinary manner with distilled water. The chemical tests were made by combining one part of virus extract with one part of the chemical at double the strength to be tested. The virus was consequently diluted only $\frac{1}{2}$, and by addition of one part of virus extract the chemical was brought to the required strength. The duration of the treatment can naturally be varied to any desired time but should not, of course, exceed the length of time the virus will live *in vitro*. In the present investigation, treatments of 1, 24, and 48 hours were tested.

EXPERIMENTAL RESULTS

Tests were performed on the thermal death point of all 4 viruses included in the experiments. The tobacco-mosaic virus was not, however, included in the aging tests on account of its extreme longevity, which rendered it impracticable to conduct the work. Trials on tolerance to dilution and resistance to chemical action were limited to the tobacco-mosaic virus and the cucumber-mosaic virus, as being sufficient for illustrative purposes. The results of the experiments are summarized in tables 1 to 11. Some incompleteness in the tables may appear to exist, brought about largely by the advisability of occasionally shifting the range of the treatments given.

Thermal death point. The thermal death point or inactivation point of ordinary tobacco-mosaic virus from tobacco is generally accepted as being close to 90° C. for a 10-minute treatment. Some variation is to be expected as a consequence of various circumstances, even though the conditions of the trials are made as constant as possible. According to McKinney (10), higher dilutions of the virus may lower the thermal death point several degrees; however, our tests have been carried on with undiluted extracts. On account of the results of previous investigations, we were at first somewhat puzzled by the failure of the tobacco-mosaic virus from tobacco to become inactivated at 90° C. in some of our trials, but we are convinced that part of this behavior may be attributed to minor modifications in technique, such as the use of the rubbing method of inoculation, and to normal biological variation.

The experiments were planned so as to submit the virus from the various hosts to temperatures well above and below the suspected thermal death point, at 5° C. intervals. If the virus in question was inactivated at a lower temperature or unaffected at a higher temperature, evidence of host influence on the thermal death point would be shown. However, it was found desirable to shift the range of temperatures in some cases, so the results presented in the tables are not always strictly comparable.

The results of studies on the influence of the host species on the thermal death point of the tobacco-mosaic virus are shown in table 1. In no case did the virus withstand a temperature of 95° C., although in several trials it withstood exposure at 90° C. Exposure at 85° C. apparently had little effect on the virus from most species tested, though in the case of Solanum miniatum, S. atropurpureum, S. melongena, and Martynia louisiana considerable inactivating action apparently occurred at this temperature. Hence, it may be concluded that in these and in most other species tested the thermal death point for the tobacco-mosaic virus is below 90° C. but that certain host species concerned may vary the thermal death point as much as 5° C. Relatively, this is not a variation of sufficient magnitude to

TABLE 1.—The influence of host species on the thermal death point of the tobacco mosaic virus^a

Source of inoculum	Temp	perature '	°C. (10 n	nin.)	Inoc.
Source of inogulum	80	85	90	95	controls
Nicotiana tabacum (tobacco)	$\frac{30}{30}$	$\frac{80}{74}$	$\frac{80}{48}$	$\frac{65}{0}$	85 85
" rustica	$\frac{20}{20}$	$\frac{15}{15}$	$\frac{20}{8}$	_	$\frac{20}{20}$
Nicandra physaloides	- <u>5</u>	$\frac{20}{17}$	<u>5</u>	$\frac{20}{0}$	$\frac{20}{20}$
Capsicum annuum (pepper)	$\frac{10}{5}$	$\frac{25}{15}$	$\frac{10}{0}$	$\frac{15}{0}$	$\frac{25}{25}$
Petunia violacea (petunia)		$\frac{15}{15}$		$\frac{15}{0}$	$\frac{15}{15}$
Physalis pubescens (ground cherry)	$\frac{5}{5}$	$\frac{20}{13}$	$\frac{5}{2}$	$\frac{15}{0}$	$\frac{20}{20}$
Lycopersicon esculentum (tomato)	$\frac{10}{10}$	$\frac{30}{29}$	$\frac{15}{1}$	20	$\frac{30}{30}$
Solanum atropurpureum	_	$\frac{20}{3}$	_	$\frac{20}{0}$	$\frac{20}{20}$
"" melongena (eggplant)	$\frac{10}{10}$	$\frac{15}{2}$	$\frac{15}{0}$		$\frac{15}{15}$
" nigrum	<u>5</u> <u>5</u>	<u>5</u>	$\frac{5}{0}$		$\frac{5}{5}$
" miniatum	$\frac{15}{15}$	$\frac{25}{5}$	$\frac{25}{0}$		$\frac{25}{25}$
Martynia louisiana	$\frac{15}{14}$	$\frac{15}{1}$	$\frac{15}{0}$	-	$\frac{15}{15}$

^a In this and in succeeding tables, upper figure represents number of plants inoculated; lower figure, number of plants infected.

be considered significant in the present state of our knowledge concerning plant viruses.

The thermal death point of the cucumber-mosaic virus, according to Doolittle (3), is 70° C., although his data indicate that the virus apparently survived heating at 70° for 10 minutes in some cases, though it did not survive 75°; consequently, the thermal death point would probably be somewhat above 70°. Our own experience with the thermal death point

of this virus, first from tobacco (7) and later from other hosts (Table 2), indicates that it behaves rather erratically, probably on account of its sensitivity to various circumstances other than the host species from which the inoculum is taken.

TABLE 2.—The influence of host species on the thermal death point of the cucumbermosaic virus

Source of inoculum	Te	mperat	ure °C.	(10 mir	ı.)	Inoc.
Source of inoculum	55	60	65	70	75	control
Nicotiana tabacum (tobacco)	$\frac{10}{6}$	$\frac{25}{23}$	$\frac{20}{11}$	$\frac{10}{0}$	$\frac{15}{0}$	$\frac{20}{19}$
" rustica	$\frac{10}{1}$	$\frac{15}{3}$	$\frac{10}{0}$	$\frac{10}{1}$	$\frac{10}{0}$	$\frac{10}{10}$
" sylvestris		$\frac{5}{0}$	$\frac{5}{0}$	5 0		5 5
" quadrivalvis		$\frac{5}{0}$	5 0	5 0	-	$\frac{5}{5}$
" glutinosa	$\frac{15}{5}$	$\frac{15}{2}$	10	$\frac{10}{0}$		$\frac{15}{5}$
Petunia violacea (petunia)	$\frac{10}{10}$	$\frac{25}{4}$	$\frac{25}{0}$	$\frac{10}{0}$	$\frac{15}{0}$	$\frac{25}{24}$
Physalis pubescens (ground cherry)		$\frac{15}{0}$	$\frac{15}{0}$		$\frac{15}{0}$	$\frac{15}{11}$
Lycopersicon esculentum (tomato)	$\frac{5}{0}$	$\frac{15}{5}$	$\frac{20}{2}$	5 0	$\frac{15}{0}$	$\frac{20}{14}$
Solanum melongena (eggplant)	$\frac{10}{0}$	$\frac{10}{0}$	5 0	$\frac{5}{0}$		10 8
" nigrum	$\frac{5}{3}$	$\frac{10}{4}$	10	$\frac{10}{0}$		$\frac{10}{8}$
Cucumis sativus (eucumber)		$\frac{15}{0}$			$\frac{15}{0}$	$\frac{15}{10}$
Apium graveolens (celery)	<u>5</u> <u>5</u>	$\frac{5}{2}$	-	$\frac{5}{0}$		5 5

It became advisable, therefore, to run some trials at a temperature as low as 50° C., although usually the range was 55 to 75°. In no case did the virus survive 75° C., and in only one questionable case did it survive 70° C. It was, however, frequently inactivated partially or entirely at 65 and 60° C., but this behavior did not appear to be correlated with any particular host

when sufficient trials were run to warrant satisfactory conclusions. A variability between 60 to 70° C. for the thermal death point of the cucumbermosaic virus may be expected, therefore, although actually it probably lies closer to the latter than the former figure.

When the experiments with the tobacco-spot-necrosis-virus complex (potato-rugose mosaic) were first undertaken, it had not yet been definitely ascertained that two separate viruses were concerned. The two types of symptoms were, therefore, referred to as the "spot-necrosis" form and the "mottle" form, and it was recognized that the former was much more susceptible to inactivation by various means than was the latter. In previous determination, the thermal death point of the mottle form, based upon extractions from tobacco and potato, was found to be 70° C., while the spot-necrosis form was destroyed at temperatures of 5 to 10° lower (8).

TABLE 3.—The influence of host species on the thermal death point of the spot-necrosis viruses^a

	\mathbf{Tem}	perature	°C. (10 n	nin.)	Inoc.
Source of inoculum	55	60	65	70	control
Nicotiana tabacum (tobacco)	15 8(3)a	$\frac{15}{0(15)}$	$\frac{15}{0(14)}$	15 0	$\frac{15}{15}$
" rustica	10	$\frac{10}{0(10)}$	$\frac{10}{0(10)}$	$\frac{10}{0(2)}$	10
" glutinosa	$\frac{5}{1(4)}$	$\frac{10}{0(8)}$	10 (8)	10	10
Petunia violacea (petunia)	$\frac{15}{10(4)}$	$\frac{15}{0(15)}$	10 0(8)	<u>15</u> 0	$\begin{array}{ c c c c c c c c c c c c c c c c c c c$
Datura stramonium (jimson weed)	5 0(5)	$\frac{10}{0(5)}$	$\frac{10}{0(4)}$	10	$\begin{array}{ c c c c c c c c c c c c c c c c c c c$
Lycopersicon esculentum (tomato)	$\frac{5}{0(5)}$	$\frac{10}{0(10)}$	$\frac{10}{0(10)}$	10	10
Solanum tuberosum (potato)	$\frac{10}{5(5)}$	$\frac{10}{0(9)}$	10 (8)	$\frac{10}{0(1)}$	10
" miniatum	$\frac{5}{2(3)}$	$\frac{10}{0(10)}$	10 0(5)	$\frac{10}{0(1)}$	10

a Mottle form in parentheses.

The range of temperatures used in the present study was 55 to 70° C. In table 3 we have attempted to show the separate action on each virus by placing the results for the mottle form in parentheses. This table again

serves to indicate that the thermal death points of the viruses have not been altered appreciably when these are taken from several distinct solanaceous host plants.

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Our previous experience with the tobacco-ring-spot virus was limited, and little was known of its properties at the time this investigation was undertaken. Recently, however, Henderson and Wingard (5) have reported the thermal death point of this virus to be between 60 and 70° C., and this agrees, in general, with our results. The data shown in table 4 indicate that it is never killed at as low a temperature as 60° C. but that

TABLE 4.—The influence of host species on the thermal death point of the tobaccoring-spot virus

	Tem	perature	°C. (10 n	nin.)	Inoc.
Source of inoculum	55	60	65	70	control
Nicotiana tabacum (tobacco)	$\frac{10}{10}$	10 10	$\frac{10}{6}$	10 0	10 10
" rustica	<u>5</u> 5	$\frac{10}{10}$	$\frac{5}{5}$	$\frac{10}{0}$	$\frac{10}{10}$
" glutinosa	<u>5</u> 5	$\frac{10}{10}$	$\frac{10}{1}$	$\frac{10}{0}$	$\frac{10}{10}$
" sylvestris	_	$\frac{5}{4}$	$\frac{5}{0}$	5	5 5
Petunia violacea (petunia)	$\frac{15}{15}$	$\frac{15}{15}$	$\frac{10}{10}$	$\frac{10}{0}$	$\frac{15}{15}$
Datura stramonium (jimson weed)	$\frac{10}{9}$	$\frac{10}{8}$	$\frac{10}{2}$	$\frac{10}{0}$	$\frac{10}{9}$
Solanum melongena (eggplant)	$\frac{5}{5}$	$\frac{15}{4}$	$\frac{10}{0}$	$\frac{15}{0}$	$\frac{15}{9}$
" miniatum	$\frac{5}{5}$	$\frac{10}{5}$	$\frac{10}{0}$	$\frac{10}{0}$	$\frac{10}{10}$

from some hosts it may be destroyed at 65° C. and that it never survived 70° C. The thermal death point, therefore, may perhaps eventually be narrowed down to a 5° range (*i.e.*, about 63 to 68° C.).

The results of the tests with these viruses on several different host species seem to show that the source of the inoculum has not caused any radical change in the thermal death point. Variations that occur might in some cases be attributed to the host species, but we do not regard them in any case as of sufficient magnitude to support the contention that this property is not sufficiently constant to warrant its utilization in a comparative

analysis of the viruses themselves. Such minor variations as do occur may, perhaps, prove to be of value for differentiation purposes where special distinctions need to be drawn.

Resistance to aging in vitro. Doolittle (3) aged the expressed juice from several species of cucurbits affected with cucumber mosaic and found that the extract was never infectious after 3 to 5 days and in most cases lost its virulence within 24 to 48 hours. Reference to table 5 shows that

TABLE 5.—The influence of host species on resistance to aging in vitro of the cucumbermosaic virus

Source of inoculum		Time	of aging		Inoc.
Source of inoculum	1 hour	1 day	2 days	3 days	controls
Nicotiana tabacum (tobacco)	$\frac{15}{15}$	$\frac{15}{1}$	$\frac{15}{0}$	_	$\frac{15}{15}$
" sanderae		<u>5</u>	$\frac{5}{0}$	$\frac{5}{0}$	$\frac{5}{5}$
" sylvestris	_	$\frac{5}{3}$	<u>5</u> 3	$\frac{5}{1}$	<u>5</u> 5
'' glutinosa	$\frac{20}{5}$	$\frac{10}{3}$	$\frac{15}{1}$	$\frac{10}{3}$	$\frac{20}{20}$
Petunia violacea (petunia)	$\frac{15}{12}$	$\frac{20}{3}$	$\frac{20}{2}$	5 0	$\frac{20}{20}$
Physalis pubescens (ground cherry)	$\frac{15}{2}$	$\frac{15}{0}$	$\frac{15}{0}$		$\frac{15}{13}$
Lycopersicon esculentum (tomato)	$\frac{15}{10}$	15	$\frac{15}{0}$	_	$\frac{15}{9}$
Solanum melongena (eggplant)	-	_	10	$\frac{5}{0}$	$\frac{10}{8}$
" nigrum	<u>5</u>	$\frac{10}{3}$	10	10	$\frac{10}{7}$
Cucumis sativus (cucumber)	$\frac{15}{10}$	$\frac{15}{0}$	5 0		$\frac{15}{12}$
Apium graveolens (celery)	5 5	$\frac{5}{5}$	$\frac{5}{0}$		$\frac{5}{5}$

similar results have been secured with this virus from various species of the Solanaceae, as tested in the present experiments. *Physalis pubescens* shows a tendency to shorten the life of the virus and *Nicotiana glutinosa* and *N. sylvestris* Speg. & Ca. perhaps a tendency to lengthen it, though in

the latter case the number of plants used was not sufficient to warrant a satisfactory conclusion. The evidence of any material influence of host species on the length of life of this virus *in vitro* is practically *nil* when compared with the remarkable longevity of the ordinary tobacco-mosaic virus.

The tobacco-ring-spot virus seems somewhat more resistant to aging than the cucumber-mosaic virus, since it is still fairly infectious in tobacco extract after 3 and 4 days aging at room temperatures (Table 6). Hen-

TABLE 6.—The influence of host species on the resistance to aging in vitro of the tobacco-ring-spot virus

Source of inoculum		Tim	e of ag	ing (da	ys)		Inoc.
Source of inoculum	1	2	3	4	5	6	control
Nicotiana tabacum (tobacco)	$\frac{25}{24}$	$\frac{25}{23}$	$\frac{25}{11}$	25 5	10 1	$\frac{10}{0}$	$\frac{25}{25}$
· · rustica	$\frac{15}{10}$	$\frac{15}{15}$	$\frac{20}{11}$	$\frac{15}{5}$	$\frac{15}{5}$	$\frac{10}{6}$	$\frac{20}{20}$
sylvestris	$\frac{5}{2}$	$\frac{5}{0}$	$\frac{5}{0}$			_	$\frac{5}{5}$
Datura stramonium	10	$\frac{10}{3}$	$\frac{10}{0}$	$\frac{10}{0}$. <u>-</u>	$\frac{10}{9}$
Solanum miniatum	$\frac{10}{8}$	$\frac{10}{5}$	$\frac{10}{0}$	5 0	$\frac{5}{0}$	5 0	$\frac{10}{10}$
" melongena (eggplant)	$\frac{15}{8}$	$\frac{20}{4}$	$\frac{20}{1}$	$\frac{15}{2}$	$\frac{20}{0}$	$\frac{15}{1}$	$\frac{20}{9}$
" carolinense	$\frac{10}{8}$	$\frac{10}{5}$	$\frac{5}{3}$	$\frac{10}{0}$	5 0	$\frac{5}{0}$	$\frac{10}{7}$
Spinacia oleracea (spinach)	$\frac{10}{3}$	$\frac{10}{1}$	$\frac{10}{0}$	$\frac{10}{0}$	$\frac{10}{0}$	$\frac{10}{0}$	$\frac{10}{1}$
Petunia violacea (petunia)	5 5	$\frac{5}{0}$	$\frac{5}{1}$	5 0	-		5 5

derson and Wingard (5) reported that this virus lost its infectivity in extract after 1 day at room temperature. This discrepancy indicates, perhaps, that in aging tests the viruses should be stored at known constant temperatures. With this virus as with the former, there is but little indication of any material change in longevity when the virus is taken from different hosts. The ring-spot virus from *Nicotiana rustica*, to be sure, shows rather strikingly longevity as compared with the same virus from

spinach, though this difference would, no doubt, be reduced if a larger number of trials were made.

TABLE 7.—The influence of host species on the resistance to aging in vitro of the spotnecrosis viruses

Source of inoculum	Time of aging (days)							
Source of mocurant	2	4	8	12	14	16	20	control
Nicotiana tabacum (tobacco)	<u>5</u> 5	$\frac{10}{7(3)^a}$	$\frac{20}{6(11)}$	$20 \over 2(10)$	$\frac{20}{2(8)}$	$\frac{10}{1(5)}$	$\frac{5}{0(4)}$	20 20
Nicotiana rustica	. 1 <u></u> 		$\frac{10}{1(7)}$	$\frac{10}{0(7)}$	$\frac{10}{0(6)}$	$\frac{10}{0(6)}$	$\frac{5}{0(4)}$	10
" glutinosa		$\frac{5}{3(2)}$	$2\frac{15}{(11)}$	$\frac{15}{0(6)}$	$\frac{15}{4(3)}$	$\frac{10}{4(6)}$	$\frac{5}{1(4)}$	15 15
Petunia violacea (petunia)			$\frac{10}{0(4)}$	$\frac{10}{0(2)}$	$\frac{10}{0(3)}$	$\frac{10}{0(4)}$	$\frac{10}{0(1)}$	10
Lycopersicon esculentum (tomato)	$\frac{5}{4(1)}$	$\frac{10}{6(3)}$	$\frac{10}{6(3)}$	5 0(1)	$\frac{10}{1(3)}$	$\frac{5}{2(1)}$		$\frac{10}{7(3)}$
Solanum melongena (eggplant)	$\frac{5}{0(2)}$	$\frac{5}{0(5)}$	$\frac{10}{0(5)}$	$\frac{10}{0(6)}$	$\frac{10}{0(4)}$	5 0(4)	$\frac{5}{0(2)}$	$\frac{10}{0(8)}$
Solanum tuberosum (potato)	$\frac{5}{3(2)}$	$\frac{10}{0(7)}$	$\frac{15}{0(5)}$	$\frac{15}{0(7)}$	$\frac{15}{0(0)}$	5 0(4)	$\frac{5}{0(0)}$	$\frac{15}{14(1)}$

a Mottle form in parentheses.

In table 7 are shown the results of aging the tobacco-spot-necrosis-virus combination. If we consider the spot-necrosis form alone, the data presented indicate some variation in behavior, especially between extracts from tobacco and from potato, these results agreeing with similar observations made earlier (8). The greatly reduced percentages of infection obtained with extracts from tobacco and other hosts after aging for several days, as compared with the controls, suggest, however, that too much significance should not be attached to this variation. The spot-necrosis form was not recovered from eggplant, even in the inoculated controls; consequently, the results secured with this host are not comparable with those secured with the other species for this form of the disease.

With regard to the mottle form alone, the data for which are shown in parentheses in table 7, there appears to be no evidence of any significant influence of the host species on the longevity in vitro.

There is admittedly room for further study of this property under more carefully controlled conditions than have obtained in the present experi-

TABLE 8.—The influence of host species on tolerance to dilution of the tobacco-mosaic virus

			Dilutio	n of viru	S	
Source of inoculum	None	1 to 100	1 to 1,000	1 to 10,000	1 to 100,000	1 to 1,000,000
Nicotiana tabacum (tobacco)	$\frac{10}{10}$	$\frac{10}{10}$	$\frac{10}{10}$	$\frac{10}{10}$	$\frac{10}{9}$	10 9
Capsicum annuum (pepper)	<u>5</u> 5	<u>5</u> 5	$\frac{10}{10}$	$\frac{10}{10}$	$\frac{10}{8}$	$\frac{10}{9}$
Petunia violacea (petunia)	$\frac{15}{15}$	$\frac{15}{15}$	$\frac{20}{20}$	$\frac{20}{20}$	$\frac{20}{20}$	$\frac{20}{20}$
Lycopersicon esculentum (tomato)	$\frac{15}{15}$	$\frac{15}{15}$	$\frac{15}{15}$	$\frac{15}{15}$	$\frac{15}{14}$	$\frac{15}{12}$
Solanum nigrum	<u>5</u>	- 5	$\frac{10}{10}$	$\frac{10}{10}$	$\frac{10}{10}$	$\frac{10}{3}$
" miniatum	5 5	5 5	$\frac{10}{10}$	$\frac{10}{10}$	$\frac{10}{10}$	$\frac{10}{7}$
Martynia louisiana	$\frac{10}{10}$	$\frac{10}{10}$	$\frac{10}{10}$	$\frac{10}{10}$	$\frac{10}{9}$	10 10

ments. The increasing evidence that environmental conditions during aging of an extract have considerable influence on longevity may account for much of the variation in the present data, since no special effort was made to maintain uniformly constant conditions. In relation to the present studies, however, it was not believed that a repetition of these trials under constant environmental conditions was necessary.

Tolerance to dilution. It might be assumed that tolerance to dilution would be more markedly affected by the host species in which the virus develops than would any other property, since both multiplication and adsorption of the virus particles might conceivably be readily influenced. However this may be, there is little evidence in support of this assumption in our dilution trials with the tobacco-mosaic virus (Table 8). Extracts from all hosts, when diluted to 1 to 1,000,000, gave good infection, except those from Solanum nigrum, which showed a fairly marked falling off at this dilution. Still higher dilutions should, perhaps, have been studied, since with the rubbing method of inoculation the limit at which infection may take place is probably considerably higher than 1 to 1,000,000. However, the data presented illustrate the significant point that this virus does not lose its infectivity at high dilutions regardless of the host species in which it develops.

TABLE 9.—The influence of host species on tolerance to dilution of the cucumbermosaic virus

			Dilu	tion of vi	rus	
Source of inoculum	None	1 to 100	1 to 1,000	1 to 10,000	1 to 100,000	1 to 1,000,000
Nicotiana tabacum (tobacco)	$\frac{10}{10}$	$\frac{10}{10}$	$\frac{10}{9}$	$\frac{10}{5}$	$\frac{15}{2}$	$\frac{15}{0}$
" rustica	$\frac{15}{15}$	$\frac{15}{13}$	$\frac{15}{4}$	$\frac{15}{1}$	15 1	$\frac{15}{0}$
Petunia violacea (petunia)	$\frac{15}{15}$	$\frac{15}{10}$	$\frac{15}{4}$	$\frac{15}{2}$	$\frac{15}{0}$	$\frac{15}{0}$
Nicandra physaloides	$\frac{10}{10}$	$\frac{10}{10}$	$\frac{10}{8}$	$\frac{10}{2}$	10 0	10
Lycopersicon esculentum (tomato)	10 8	10 10	10 8	$\frac{10}{0}$	10	$\frac{5}{0}$
Solanum melongena (eggplant)	$\frac{10}{8}$	$\frac{10}{5}$	$\frac{15}{6}$	$\frac{15}{2}$	$\frac{15}{0}$	
" nigrum	$\frac{5}{5}$	$\frac{5}{5}$	$\frac{10}{3}$	$\frac{10}{4}$	$\frac{10}{0}$	$\frac{10}{0}$
Apium graveolens (celery)	$\frac{10}{10}$	10 10	10 8	$\frac{10}{3}$	10	10

The cucumber-mosaic virus is incapable of causing infection at as high dilutions as the tobacco-mosaic virus, even if the rubbing method of inoculation is used. Doolittle (3) secured infection at 1 to 10,000 from mosaic-cucumber extracts, using another method of inoculation. It is interesting to note that, while the present trials (Table 9) show some instances of infection at 1 to 100,000, a marked falling off occurred at 1 to 10,000. The rubbing method of inoculation, therefore, does not appear to be so effective with this virus, in comparison with other methods, as in the case of the tobacco-mosaic virus. Considering the number of plants used in the dilution tests with the cucumber-mosaic virus, the results secured with the various hosts are very similar.

Resistance to chemicals. The high resistance of the tobacco-mosaic virus to various chemical treatments was demonstrated by Allard (1). Doolittle (3) made similar though less extensive trials with the cucumbermosaic virus and came to the conclusion that the tobacco-mosaic virus was apparently slightly more resistant to chemicals than the former. Extensive studies of a wide range of chemicals on a variety of viruses would, no doubt, be productive of some very interesting results. Our previously re-

TABLE 10.—The influence of host species on the resistance to chemicals of the tobaccomosaic virus

	Nitric aci	d 1 to 200	Ethyl alc	ohol 50%	Inoc.
Source of inoculum	24 hours	48 hours	24 hours	48 hours	control
Nicotiana tabacum (tobacco)	$\frac{25}{25}$	$\frac{15}{15}$	$\frac{25}{25}$	15 15	$\frac{25}{25}$
Capsicum annuum (pepper)	$\frac{20}{18}$	$\frac{15}{15}$	$\frac{20}{20}$	$\frac{15}{15}$	$\frac{20}{20}$
Petunia violacea (petunia)	$\frac{15}{15}$	$\frac{15}{15}$	$\frac{15}{15}$	15 15	15 15
Physalis pubescens (ground cherry)	15 13	$\frac{15}{15}$	$\frac{15}{15}$	15 15	$\frac{15}{15}$
Nicandra physaloides	$\frac{15}{15}$	$\frac{15}{15}$	<u>5</u> -5	$\frac{15}{14}$	$\frac{15}{15}$
Lycopersicon esculentum (tomato)	$\frac{20}{20}$	$\frac{15}{15}$	$\frac{20}{20}$	$\frac{15}{15}$	$\frac{20}{20}$
Solanum atropurpureum	<u>5</u> 5	$\frac{15}{15}$	<u>5</u>	$\frac{15}{15}$	$\frac{15}{15}$
nigrum	<u>5</u>	$\frac{5}{5}$	5	<u>5</u>	5 5

ported limited trials on this subject (7), together with the results secured in the present studies (Tables 10 and 11), show rather conclusively that the tobacco-mosaic virus and the cucumber-mosaic virus are very different with respect to their resistance to the action of nitric acid and alcohol and that they cannot be placed in the same category in this respect. In order to arrive at the approximate lethal points for each virus, entirely different concentrations of chemicals or times of exposure would need to be used. However, it is again believed that the data presented in tables 10 and 11 are sufficient to indicate that the host species from which the respective viruses were obtained have neither tended to increase nor decrease the resistance to the chemicals used and that this particular property as well remains strikingly constant in this respect.

DISCUSSION

The studies on the influence of host species on the properties of plant viruses were at first undertaken with some hesitancy as to their value in view of the rapidly increasing evidence that plant viruses are specific entities that cannot be changed over from one known form to another.

TABLE 11.—The influence of host species on the resistance to chemicals of the cucumber-mosaic virus

Source of inoculum	Nitric aci	d 1 to 200	Ethyl ald	ohol 50%	Inoc.	
Source of moedium	1 hour	24 hours	1 hour	24 hours	(1 hour)	
Nicotiana tabacum (tobacco)	30 2	$\frac{30}{0}$	$\frac{30}{4}$	$\frac{30}{0}$	$\frac{30}{25}$	
rustica	10	$\frac{10}{0}$	$\frac{10}{0}$	$\frac{10}{0}$	$\frac{10}{9}$	
" glutinosa	5 0	5 0	$\frac{5}{0}$	$\frac{5}{0}$	$\frac{5}{5}$	
Petunia violacea (petunia)	$\frac{15}{0}$	10 0	$\frac{15}{0}$	$\frac{15}{0}$	$\frac{15}{14}$	
Physalis pubescens (ground cherry)	$\frac{15}{0}$	$\frac{15}{0}$	$\frac{15}{0}$	$\frac{15}{0}$	$\frac{15}{13}$	
Lycopersicon esculentum (tomato)	$\frac{20}{0}$	$\frac{20}{0}$	$\frac{20}{0}$	$\frac{20}{0}$	$\frac{20}{19}$	
Cucumis sativus (cucumber)	$\frac{15}{0}$	$\frac{15}{0}$	$\frac{15}{0}$	$\frac{15}{0}$	15 8	

However, as long as any belief remained that host species could radically change the properties of a virus or that these properties cannot be satisfactorily compared, such beliefs constituted a challenge to the progress of any scheme of differentiation and classification of plant viruses utilizing properties as one criterion.

The experimental results shown in tables 1 to 11 present evidence as to the constancy of these properties in their broader aspects. It may be pointed out that Walker's (11) conclusions were drawn at a time when the specificity of viruses was not generally recognized and that McKinney's (10) conclusions with respect to hosts species are apparently not based on data of his own but rather on Walker's results. Until further data are presented to support the contention that virus properties are not reliable comparative criteria of the specific viruses themselves, it seems logical to utilize these simple and convenient means for purposes of differentiation, together with any other characteristics that may be of value. Refinement in technique is, of course, desirable and may be quite necessary for finer distinctions between the ever-increasing numbers of viruses now being recognized.

The results of the present investigation suggest that property studies might quite as well be carried on with one susceptible plant species as another and still be comparable with previous work. However, there are

some manifest advantages in the use of certain hosts for such studies. Consequently, in working with new or previously described viruses found on new or old hosts, it may still be advisable to transmit the virus, whenever possible, to some convenient and common host plant for purposes of property studies.

In conducting the experimental trials described, many interesting features other than those already discussed were noted, which merit further study. Some of these are closely related to the problem under consideration and are worthy of mention in the present connection.

The influence of the "source of inoculum" on the relative ease with which a virus may be recovered from a given host has been a moot point for some time. Previous experience had indicated that certain hosts showing distinct symptoms as a result of inoculation with a virus failed partially or completely to yield infectious extracts in the ordinary manner. In certain cases this behavior apparently may be influenced by such factors as age and vigor of the host plants, environmental conditions, or localization of symptoms. Following repeated trials, evidence of complete failure to secure infection from certain hosts has been gradually breaking down. However, some evidence of partial failure may be noted in the present investigation in the inoculated controls with the cucumber-mosaic virus (Tables 2, 5), and the ring-spot virus (Table 6), although this behavior apparently has not affected the properties of the virus as such.

Not only may the ease of recovery of a virus be influenced to a considerable extent, but the incubation period and the intensity of the symptoms are apparently affected in some cases, although only in rare cases did we apparently secure actual modification (attenuation) of the virus itself, namely, with passage of tobacco mosaic through Martynia. Aside from these cases, the influence of host species on symptoms appeared to be temporary, and it is not at all clear that these influences were actually due to the host species involved rather than to the condition of the host or virus itself.

Judging from the differences in relative susceptibility of the various hosts used, approaching immunity in some cases, it may logically be reasoned that the viruses are actually subjected to very different circumstances within the various hosts, and the fact that they remain as constant as they do argues strongly against any theory that the virus results in whole or in part from any activity or product of the host itself but that it is rather an entity quite foreign to the host concerned.

SUMMARY

1. The thermal death point, resistance to aging in vitro, dilution tolerance, and the resistance to certain chemicals of the viruses of ordinary

tobacco mosaic, cucumber mosaic, tobacco ring spot, and tobacco spot necrosis (potato-rugose mosaic) have been compared in extracts from several species of host plants.

- 2. The experimental results show that the host species in which the viruses developed did not radically influence the constancy of the properties of each virus. Some minor influences were noted that are hardly to be regarded as of sufficient magnitude to be of actual significance.
- 3. The contentions that have previously been made in the literature that plant viruses may be fundamentally changed by the host plant affected and that the properties of viruses cannot be adequately studied in a comparative way with the ordinary technique are consequently not supported by the results secured.
- 4. It is believed that the properties of artificially transmissible plant viruses offer one of the most convenient and reliable criteria for their isolation, differentiation, and classification.

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A CENTRALIZED SCAB-SPRAY SERVICE

A. L. PIERSTORFF (Accepted for publication February 11, 1932)

Pioneer work in spray-information service was started in New York State during the World War.¹ Food production and conservation were imperative. To aid in this noble cause an experiment in better timing of apple-scab sprays was initiated in a few New York counties. From this successful venture, the idea of giving timely spray information to growers has spread to other important eastern apple-producing States.

Under a decentralized system it was extremely difficult for the sprayservice supervisor to keep in constant touch with the field men, especially when some men traveled over several counties. It was equally difficult for the county agent to keep in touch at all times with the field man of his Sometimes growers would call the county agent and ask whether it was time to spray. Perhaps, the grower would add that his neighbor was spraying or that his neighbor, who might be in a different county, had received word to spray. This was an embarrassing situation for the county agent, who, with many projects to supervise, might not know what to recommend and then would call headquarters. Not knowing the stage of bud development or what the field man had said, headquarters frequently was helpless. The result was constant confusion during the early season. An additional handicap was that experienced field men were not always available for a few months' service, and there was great risk in putting an inexperienced man in charge of such work. With a decentralized plan, the success of a spray service in any given locality depends to a large extent upon the judgment of the local field man.

Improvements in communication equipment have greatly aided in making a centralized spray service possible. Telephone, telegraph, and radio all play an important rôle. Since 1925 the Ohio spray service has been expanded and modified to fit more nearly Ohio conditions.² The greatest concentration of fruit in Ohio is found in those counties that are located on the rim of the State and that border the Ohio River or Lake Erie. However, in each of the 88 counties there are some commercial growers whose major incomes are derived from the sale of fruit. How adequately to advise such growers was a real problem in the early days of the spray

¹ Barrus, M. F. The organization of a special extension service in New York State. U. S. Dept. Agr. Off. Coop. Ext. Work. Ext. Path. 1⁽²⁾: 4-10. 1923. (Mimeographed.)

² Appreciation is hereby acknowledged to Dr. H. C. Young, of the Ohio Agricultural Experiment Station, and his staff and to Dr. W. G. Stover, of the Ohio State University, for initiating and aiding in developing the spray service.

service. The fact is such scattered growers were entirely ignored. The shift in Ohio has been gradual from a decentralized system involving the use of 8 or 10 highly trained men for a relatively short time in the spring to a centralized plan. In this latter plan, one individual makes recommendations for the time of application of the various sprays for the entire State, in so far as diseases are concerned. Consultation with other patholo-

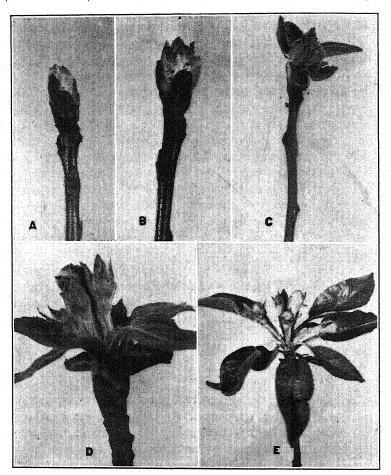


Fig. 1. Stages in apple-bud development. A. Silver bud. B. Delayed dormant. C. Early prepink. D. Prepink. E. Pink.

gists is naturally frequent. Under this plan it is possible for every fruit grower in the State to receive timely spray information.

Three factors are taken into consideration before a decision upon the time of application is reached. The first is the stage of fruit-bud develop-

ment; the second, the percentage of mature ascospores of the scab fungus; and the third, the probable weather for the next 2 or 3 days.

Information on fruit-bud development is obtained from distant points by telegraph or telephone. About 25 cooperators send telegraphic reports to the central office 3 times each week, on Monday, Wednesday, and Friday mornings. If development is extremely rapid, a daily wire is sent to the central office. A small booklet containing 8 colored pictures illustrating the different stages of bud development, from silver bud to full pink, is furnished each cooperator (Fig 1). With this as a guide and following the terminology used in the booklet, the stage of bud development of 3 to 5 common commercial varieties is wired to the central office. In addition to bud development, information on the number of hours of continuous



Fig. 2. Areas used in apple-scab radio broadcasting.

rainfall and on the progress of spraying in the community is included in the messages. These reports begin when the earliest blooming variety approaches the delayed dormant stage and cease when the latest blooming variety is in the late pink stage (Fig. 1, B and E, respectively).

Following is one of the many messages received last spring from a fruit-grower cooperator: "April 26, 1931: 'Duchess and early varieties pink. Baldwin early pink closely approximating pink. Rome late prepink. Cold weather has brought the buds very close together. Rained continuously last 48 hours.' Signed C. H. Stokes."

The State is divided into 17 broadcasting areas, the boundary lines of which was determined by observing fruit-bud development (Fig. 2). These boundaries are changed as our knowledge of bud development increases. In each broadcasting area there is at least one cooperator. In

some areas there are several. In most of these areas, some cooperators are fruit growers, some are county agricultural agents, and two are vocational agricultural teachers. In the hilly section it is necessary that a cooperator state both hill and valley conditions. Along Lake Erie, development at different distances from the lake must be given. As rapidly as these reports are received they are transferred to a large State outline map that has been divided into broadcasting areas.

A second set of cooperators—in some cases the same individuals who send telegraphic reports—send scabby apple leaves of the previous season to the central office 3 times a week. Many growers include samples of new buds with the old leaves. These leaves are usually received and examined within 30 hours after they are collected. A supply of self-addressed, stamped envelopes is furnished each cooperator.

After boiling the leaves in a concentrated solution of either sodium or potassium hydroxide to remove most of the opaque organic matter, the perithecia of the scab fungus can be speedily and accurately counted. From each collection one or two leaves, without boiling, are placed in a humid chamber and the spore discharge noted on the following day. In the fall of the year each collaborator is instructed to collect several hundred scabby apple leaves and place them on the ground under an apple tree near his home with a piece of wire netting over them in order to be certain that he will be able to find old scabby leaves in the spring.

A record of ascospore development is kept, for each cooperator. The details of this record are shown in table 1. The most important columns are the percentage of mature spores, or nearly mature, and, after petal fall, the percentage discharged. By receiving leaves regularly triweekly an accurate record of ascospore development for each area of the State is obtained. It will be noted that a few ascospores were mature for a month before any discharge was observed. There were no heavy rains in that section of the State from which the leaves were received during that interval. It has frequently been observed that a light rain will not cause discharge of many ascospores if the old leaves have been thoroughly dried for a week or 10 days immediately before the rain, even though the spores appear to be mature. From the 18th to the 28th of April, continued rains occurred and 60 per cent of the spores were discharged.

Three samples of leaves are received on successive days about one week after petal fall. From these samples an indication of the potential load of spores still to be discharged can be obtained. The higher this percentage, the greater is the need for 10 days' to 2 weeks' spray.

Special weather forecasts giving the probable temperature, precipitation, and wind velocity for the next 2 or 3 days are received daily. All 3 of these factors must be taken into consideration when making spray recommendations.

TABLE 1.—Ascospore development record, 1930

Address-Van Wert, Ohio

Name of grower-Dean Clippinger

County-Van Wert

Spores discharged	$Per\ ct.$	00	0	000	0	0 (O 10	10-15	25–30	09		65	65 65
Mature spores	Per ct . $1-3$	1-5 -3	4-6	3-4	1 d	10	x x x	35	35	20		20	202
2-cell hyaline spores	$Per\ ct.$ 5	10	ıc	8-10	12.	50	× 17	14	14	12			1t
Hya- line spores	$\frac{Per\ ct.}{20}$	20 12	15	15	12	10	~ 9	က	ന	4		ge in	r—some —abundan
Asci cut out	Per et. 30	32 30	40	30	40	70	70	7.5	06	92		re discharge in	st chambe
Proto- plasm granular	Per et. 70	65 70	09	20	00	30	0000	25	10	∞		Spe	moist
Peri- thecia full- size	Per et. 30	35 30	40	30	40	20	70	75	06	65			
No. peri- thecia crushed	15	16 15	18	∞ ç	15	25	20 25	122	15	20		20	24 18
No. peri- thecia exam- ined	7.0	75 90	08	75	0 6 0 6	100	95	100	95	95	ploom	70	75
No. of leaves	4	טי טי	4	, O.	∞ 41	4	ധ 4	ı ro	10		trees in b		10
Date exam- ined	Mar. 18	19 25	Apr. 1	101	~ ∞	10	17	55	56	May 1	Apple	16	17
Date col- lected			31										16 17
No. of sample	7	12 34	49	64		150	164	208	215	225		251	254 260

a Seven, 8, and 9 days, respectively, after petal fall, to aid in determining the necessity of a 10-14 days' spray.

To illustrate clearly the details of the spray service, the procedure during a typical April day is chronologically recorded.

Shortly after 8:00 a.m. telegraph and telephone messages begin to arrive. By 9:45 all the reports have been received and have been transferred to a State outline map. About 10:00 a.m. the special weather forecast is received. Further consultation by telephone with the local Weather Bureau or pathologists at Wooster may be required. The percentage of mature ascospores in various sections of the State is noted. A radio talk of about 600 words is then prepared, giving the bud development for the various areas and suggestions for spraying or for withholding the spray. Differences in varietal bud development and variations in hill and valley orchards must be considered. By 11:15 the talk is given to the local telegraph operator, who has his lines cleared and is directly connected with the simplex machine in the distant radio station of WLW, Cincinnati, and with WTAM, Cleveland. As rapidly as he sends the message it is received on the ticker tape in Cincinnati and Cleveland. About 15 minutes are required to transmit the message, the accuracy of which depends entirely upon the sender. The simplex machines are electrically controlled, and, by checking the message as it emerges from the sending machine, errors are reduced to a minimum.

The county agricultural agents located in Cincinnati and Cleveland put the spray message on the air about 11:50 a.m. and 12:25 p.m., respectively. The actual time of broadcasting is arranged with the broadcasting-station staff each spring, and every fruit grower is notified by mail when to tune in. From 3 to 5 talks are given each week in addition to the weekly summary talks given over WEAO, the local University station.

Preceding the time for each spray or group of sprays, a mimeographed letter is sent to each fruit grower on the county agents' mailing lists. These letters are prepared jointly by the extension plant pathologist and the extension entomologist. They are sent to the county offices in bulk. After affixing his signature, the county agent mails one to each grower. These letters contain all the information necessary for applying a spray, except the time of application for those sprays that are timed by radio. When the critical scab period has passed the extension entomologist takes charge of the radio and broadcasts weekly until about July 1. Information on the severity of scab or on the necessity of applying Bordeaux for blotch and Brooks' spot is included in his weekly broadcasts.

Since human nature is fundamentally alike the world over, some Ohio growers usually feel that certain sprays are not correctly timed for their particular orchards. Granting that they may have some grounds for their views, nevertheless one usually finds that there are other contributing factors within the grower's control to account for their spray failures. To check on these points certain growers were formerly requested to spray a

portion of their orchard exactly as directed by the spray service. The grower was given a chart upon which was kept an accurate record of his spraying activities. Counts on insect and disease injuries were made by a representative of the extension service at harvest time. The results were so gratifying that more and more growers requested forms upon which to keep spray records until at the present time not all the orchards can be visited in the fall where such records are kept. To be sure, not all these growers will follow in detail every recommendation made by the spray service. This is not desired, because not all growers have exactly the same problems.

The average of the results in the orchards where spray recommendations were generally followed are given in table 2. The average of 95.75 per cent

TABLE	2.—Four years'	results of	apple spraying	where	$spray\mbox{-}service$	recommendations	
			were followed				

Year	Number of orchards	Times sprayed	Percentage of scab	Percentage of other diseases	Total percentage	Percentage disease- free fruit
1928	21	6.1	4.5	0.4	4.9	95.1
1929	27	7.1	5.26	0.45	5.71	94.29
1930	98	6.0	2.37	0.01	2.38	97.62
1931	94	5.5	2.0	2.0	4.0	96.0
Average		6.2	3.53	0.71	4.25	95.75

disease-free fruit is fairly high for a group of approximately 100 farmers. The commercial grower cannot afford to have absolutely clean fruit. The last several per cent costs too much to produce. If 95 per cent of the fruit is absolutely disease-free, at least 97 per cent is commercially disease-free. Insect injuries average about the same as those for diseases, so that the commercial grower would have 90 per cent of his fruit free from blemishes. Culls due to disease or insect injuries would not exceed 7 per cent.

There are several advantages in using the radio for making spray recommendations. Different sets of conditions can be outlined and the intelligent grower can then apply those recommendations that best fit his needs. Studying his own orchard problems, he can frequently save one or two sprays during years of light scab-fungus infection and can apply more sprays during severe seasons. It makes it possible for the intelligent grower to produce a clean crop year after year by using a minimum number of sprays.

When necessary, radio talks may be given every day for 5 minutes or longer and an opportunity is afforded for teaching the "why" of spraying in addition to the "when" and the "how." If the growers see the reason

behind the recommendation, they will be more faithful in their efforts. Sometimes weather conditions are uncertain and it is impossible to accurately forecast a rain 3 or even 2 days in advance. Growers can be warned that conditions are uncertain. By outlining what will happen in their orchards if the impending rain occurs on unprotected foliage and then by mentioning the other side of the story, that if it does not rain the spray is wasted, the growers can make their own decisions based upon experience. It is one of the purposes of extension teaching to aid the farmers in making correct decisions. On the following day, when more accurate information on the threatening rain is available, growers located in sections where fruit buds are unprotected can be urged to spray or to withhold the application. By no other means than by the radio is it possible to reach all interested growers every day. It is almost equal to a visit to their individual orchards.

Time is an all-important factor in apple-scab control. A few minutes mean many dollars to 4,000 fruit growers. They are quick to take advantage of the radio, as is evidenced by the letters and telegrams they send to the central office during the spray season. Many request a reply the following day by radio. If radio spray service is to be successful, the broadcaster must be familiar with orchard operations and the station must be powerful enough for the talks to be received without fail on farm sets. Ohio is fortunate in this respect in that two 50,000-watt stations are located in opposite ends of the State.

One of the criticisms of any spray-information service is that year after year the same type of information is outlined for the growers. This must necessarily be the case because growers do not have the physical equipment with which to study ascospore development; even if they had, only a few would interpret the facts correctly. They can observe fruit-bud development accurately in their own orehard, and this they must do in order to apply sprays intelligently. Three-day weather forecasts are not available to them and such forecasts require considerable interpretation before using.

The spray service in Ohio has taught the fruit growers that sprays must be applied promptly. The service has had a marked influence on thoroughness of spraying and has made it necessary for the individual to study critically his own problems. By striving to cover their orchards in as short a time and as thoroughly as possible many orchard men have effected economies in spraying as high as 50 per cent. Since spraying is approximately 30 per cent of the total growing cost of an apple crop, this is a sizable item. These advantages coupled with the increased control obtained by better timing have made the spray service very popular.

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VERTICILLIUM WILT OF COTTON IN MISSISSIPPI

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In the fall of 1930 a hitherto unreported disease of cotton was discovered in Mississippi. This discovery was entirely fortuitous and occurred in connection with operations designed with regard to an entirely different trouble. A collection of 19 cotton stalks, showing internal discoloration supposedly symptomatic of Fusarium wilt, made on some experimental plots at the Delta Branch Experiment Station at Stoneville, Mississippi, on being cultured, gave no growth of Fusarium vasinfectum, as was expected. On the other hand, each and every stalk gave rise to a pure culture of a species of Verticillium that agreed in all essential morphological characters with the descriptions of V. alboatrum R. and B. These cultures were compared with one of that organism secured from C. D. Sherbakoff and were found to be in agreement. Sherbakoff's culture was made from cotton from Lake County, Tennessee, in 1928.

Verticillium alboatrum was first recorded on cotton in 1914 by Carpenter (1), who found it causing wilt symptoms on 2 plants at Arlington, Virginia. No descriptions or experimental data were recorded by Carpenter, but he expressed the opinion that V. alboatrum might be responsible for some of the damage to cotton that is now attributed to Fusarium vasinfectum. In 1918 (2) he briefly reported successful inoculation of cotton plants with Verticillium secured from okra. In 1921 (3) and 1922 (4) Bewley reported similar success on cotton with Verticillium from tomato.

In 1928 Sherbakoff³ briefly reported the discovery of the disease in cotton on the gumbo types of soil of the Mississippi River bottom in Tennessee. In 1929 (5, 6), in other brief articles, he records its discovery in the same type of soil on the opposite side of the Mississippi River, in Arkansas. Aside from citations of the above-mentioned papers by Rudolph (7, p. 306) in 1931 in his extensive treatise on Verticillium hadromycosis, no other reference to a Verticillium on cotton has come to the writers' attention.

Published descriptions of the disease as it appears on cotton are very meager and unsatisfactory. Bewley (3, 4) merely reports that the plants that he inoculated were stunted and that desiccation of the leaves occurred

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³ Sherbakoff, C. D. Wilt caused by *Verticillium alboatrum*. U. S. Dept. Agr., Bur. Plant Indus., Plant Dis. Rptr. Sup. 61: 283-284. 1928. (Mimeographed.)

35 days after inoculation. Carpenter (2) reported that 80 per cent of 20 plants inoculated showed symptoms of wilt in 14 days. He further states that Fusarium wilt and Verticillium wilt are indistinguishable except by cultural methods. Sherbakoff (5), in his brief article in Phytopathology, makes an attempt to distinguish between the two, but his descriptions are contradictory. In an acquaintance with and observation of the disease over a period of 2 years, the writers have been unable to discover any difference in symptoms that has been consistent enough to allow them to distinguish between the 2 wilts in the field without further recourse to laboratory methods for confirmation. At present the writers can only state that the symptoms of the Verticillium wilt as observed are identical with those of the wilt caused by Fusarium vasinfectum as those of the latter organism are manifested on soil types on which the Verticillium has been observed. A further study of the symptomatology of the Verticillium wilt that is at present contemplated may, however, reveal differences.

Although the Verticillium wilt was first discovered in Mississippi in the fall of 1930, the plots on which it was found have been used continuously since 1928 for the testing of varietal resistance of cotton to wilt. The wilt against which resistance was being tested was supposedly that caused by Fusarium vasinfectum. This testing work was begun by D. C. Neal in the spring of 1928 and was turned over to the senior writer in the fall of that year. Heavy, artificial inoculation of the soil at planting time with cultures of F. vasinfectum grown on wheat bran and mixed with sand for distributional purposes was resorted to by Neal in 1928 and by the writers in 1929 and 1930. It was, therefore, assumed that the large amount of wilt that developed was due to that organism. This supposition was rendered still more plausible by the fact that the culture of F. vasinfectum that was used in making the soil inoculum for the plots and that the senior writer found in stock when he took over the project bore a label indicating that it had been isolated by Neal from a plant in those same plots the preceding year.

However, examination of the records for the 3-year period from 1928 to 1930 revealed that varieties of cotton that were showing resistance to Fusarium wilt in other parts of the State were not responding similarly in these plots. The idea at once occurred that this difference might be due to the presence in these plots of a strain of F. vasinfectum that differed in pathogenicity from those strains present in the plots in other parts of the State. It was to test this idea that diseased stalks were collected and isolations made in 1930, with the resultant discovery of Verticillium alboatrum as the causative agent rather than a new pathogenetic strain of F. vasinfectum. In addition to these 19 isolations made in 1930, 40 more were made from diseased plants in these plots in 1931 and from not a single one

has a culture of F. vasinfectum been secured. By far the greater number have yielded pure cultures of V. alboatrum. It would seem, therefore, that F. vasinfectum has not been able to persist in this soil.

Careful actual wilt counts are available from these plots for the past 4 years, 1928 to 1931. The average infection for these years is as follows: 1928, 62.82 per cent; 1929, 80.00 per cent; 1930, 35.53 per cent; 1931, 17.66 per cent. It will be seen that there is throughout this period a wide yearly variation in the percentage of actual wilt infection as revealed by counts at the end of the season. The higher percentages in 1928, 1929, and 1930 may possibly be accounted for by the artificial soil inoculation with Fusarium vasinfectum that was made in those years. No artificial inoculation was resorted to in 1931. Had this inoculation had an appreciable effect, however, one would have expected it to be revealed in an increased wilt count on the varieties that elsewhere in the State have shown a high degree of susceptibility to Fusarium and, conversely, in a lower wilt percentage in the varieties resistant to Fusarium. Careful analysis of the figures for those years does not yield an impression that they are the result of a combined effect of the Verticillium naturally present and the Fusarium that was introduced. The lower infection in 1930 may have been due to the very severe and extended drought of that year, serving to restrict the activities of one or both of the pathogens. The still lower infection count in 1931 may have been due to the dying out of the Fusarium possibly present in previous years; or, on the other hand, it may have been the result of environmental factors unfavorable to the Verticillium. Sherbakoff* states that it is the opinion of the growers in Lake County, Tennessee, where he first discovered the disease, that it occurs there irregularly, or about once in 3 or 4 years. The uniformity of infection on all varieties in 1931 when 60 isolations failed to reveal any Fusarium and in the preceding 3 years leads one to believe that Verticillium alone is chiefly responsible for the wilt on these plots.

Forty varieties of cotton in all have been tested for wilt resistance on these plots in this 4 year period, 1928 to 1931. Not all, however, have been in the test for the full period. In table 1 is shown the average actual wilt infection as recorded at harvest time for each variety that was represented in the tests for at least 2 years.

The varieties were planted in 107 foot rows and in 4 replications each year. Wilt counts were secured by cutting all stalks in each row and recording as infected all that showed internal discoloration. The table reveals that for any one year the variation in percentage of infection is so slight that no indication of resistance can be deduced for any variety tested

⁴ Loc. cit.

TABLE 1.—Actual wilt infection percentages, 1928-1931, on 18 varieties of cotton

	Average percentage infected					
Variety	1928	1929	1930	1931		
Cleveland 54	59.6	75.4	58.75	16.25		
Miller 610	65.5	84.2	53.00	16.25		
Lightning Express	68.7	85.4	34.00	17.25		
Dixie Triumph	61.8	80.3	42.75			
Super Seven	47.5	79.1	18.25			
D. & P. L. 4–8		66.3	23.50	13.25		
Missdel No. 2		88.1	47.50	11.75		
D. & P. L. 4128A-21-32	71.0	75.7				
Delfos 910	74.2	89.1				
Express 121	57.7			17.50		
Willis Triumph		77.6	37.75			
D. & P. L. 5242A-310-46		62.9	25.00			
Cook 1010		81.6	38.75	***************************************		
D. & P. L. 6		82.3	22.75			
D. & P. L. 10			33.00	16.25		
Express 121-41077			29.00	10.00		
Missdel No. 3			34.75	21.75		
Missdel No. 1	********		34.75	23.75		
Average infection for above varie-						
ties	63.25	78.69	35.55	16.40		
Average infection total number						
tested	62.82	80.00	35.53	17.66		

or, at least, not on the basis of actual wilt infection counts at the end of the season.

In spite of the fact that the disease was discovered quite late in the fall of 1930, a brief scouting trip was made through a number of counties located chiefly in the Mississippi Delta to secure data regarding its distribution and prevalence. Collections of diseased cotton stalks were made from 21 properties located in 11 counties. Seven of these yielded cultures of the Verticillium, 10 yielded Fusarium only, and the other 4 gave neither Fusarium nor Verticillium. On account of the late date at which the collections were made many of the stalks collected were practically dead and contaminating saprophytic organisms had attained such a foothold that they outgrew and obscured the cultures of organisms that were responsible for the internal discolorations.

The counties in which Verticillium wilt was located in this brief survey were Bolivar, DeSoto, Tunica, and Washington. All of these are located in the Mississippi Delta and, in fact, immediately adjacent to the Missis-

sippi River. The soil in all cases was a heavy, sedimentary loam of the type usually termed gumbo.

In 1931 this survey was extended. County agents, Smith-Hughes teachers, State Plant Board inspectors, and other agricultural workers throughout the State were solicited to send in specimens of cotton plants showing symptoms of wilt. Internal discolorations of the woody tissues of the stem and taproot were in most cases taken as the diagnostic symptoms. Two or 3 brief scouting trips by the writers served to supplement somewhat this cooperative survey carried on by correspondence. All specimens received or collected were cultured immediately by the junior writer.

The total number of collections sent in by cooperators was 69. Twenty more were made by the writers, making a total of 89 in 1931. Each collection consisted of from 5 to 10 or more plants and each plant was cultured in a single Petri dish. Thirteen of the 89 collections cultured yielded cultures of Verticillium only, 73 showed Fusarium alone, and 3 yielded both Fusarium and Verticillium. Verticillium, therefore, was present in 16 and Fusarium in 76 of the 89 collections made. The map, figure 1, shows the distribution of these infections in the State.

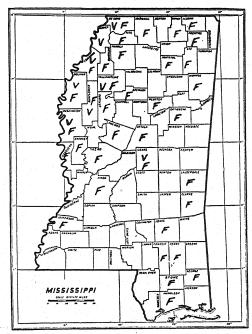


Fig. 1. Distribution of Fusarium vasinfectum and Verticillium alboatrum as revealed by the survey. F, Fusarium. V, Verticillium.

The collections cultured represented 37 of the 82 counties in Mississippi. Fusarium cultures were secured from plants from 36 of these 37

counties and Verticillium from 8. The total number of plants from which cultures of either Fusarium or Verticillium were secured was 706. Numerous others yielded no cultures of either or were overgrown with other contaminating organisms and were discarded. Six hundred and twenty-six of the 706 plants yielded Fusarium and 80 gave cultures of Verticillium. The counties in the State in which Verticillium is now known to occur are DeSoto, Tallahatchie, Bolivar, Sunflower, Coahoma, Tunica, Washington, and Leake. All of these are located in the Delta, with the single exception of Leake, which is in the central part of the State.

The infection in Leake County occurs on rich, loamy soil in riverbottom land. One of those in Tallahatchie County is in the hill section outside the limits of the Delta but is on bottom land of a rich, loamv nature. The others are all on typical Delta soils, mostly of the gumbo type. So far, the disease has not been found on any of the lighter or sandy soils of the State. Sherbakoff (5) states that he has found it only on the gumbo river-bottom soils of Tennessee and Arkansas. Rudolph (7), speaking of Verticillium in general as it occurs on a large number of host plants, states that opinion is sharply divided as to which soil types are most conducive to the development of the disease and the propagation of the fungus. He states, however, that in California Verticillium hadromycosis, incidentally on crops other than cotton, has been observed in severe epidemic form on soils varying from a light, sandy loam to a heavy clay and to adobe types. Bewley (3, 4) considers clay soils as favorable to the development of the disease on tomatoes. Van der Lek (8) also regards clay as well as sedimentary soils as favorable to the propagation of the fungus.

Observations made in Mississippi, though too few in number and superficial in character, tend to indicate that the heavier sedimentary and alluvial soils are more favorable to the disease than are the lighter, sandier types. This is, in fact, rather definitely indicated in the experimental plots at the Delta Branch Experiment Station on which the disease was first discovered in the State. The soil in one limited area near the central part of the plot is lighter in color and texture than the remainder and contains a larger proportion of sand in its composition. On this area the cotton during the last 2 years was observed to be somewhat shorter and of less vigorous growth and the percentage of plants showing infection was also considerably lower than on the heavier soils on either side.

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BLIGHT OF PEPPERS IN FLORIDA CAUSED BY PHYTOPHTHORA CAPSICI

GEORGE F. WEBER (Accepted for publication January 25, 1932)

During the winter-growing season of 1930-31, large, bearing pepper plants of the Ruby King and California Wonder varieties growing in the Homestead area were found affected with a blight caused by Phytophthora capsici Leonian. This is the first time that this fungus has been collected in Florida and the second report of its collection in continental United The infected areas were small and confined to one section of the field, apparently resulting from the use of diseased seedlings originating from infected seed. The pathogen was isolated and identified through comparisons with Phytophthora species occurring in Florida, with a culture of the original species supplied by Leonian, and through artificial inocula-This identification was verified by Tucker, who had previously collected and determined this parasite from Puerto Rico. In addition to the establishment of the occurrence and pathogenicity of this parasite on peppers in Florida, the purpose of this paper is to present certain information concerning the symptoms of the disease in the field in addition to those given by Leonian.2 as it occurred in New Mexico.

THE DISEASE

Symptoms. Diseased pepper plants in Florida showed infected stems, branches, fruit, and leaves. Infected stems were often girdled at the soil line, which resulted in a sudden wilting and death of the entire plant. The girdling lesions extended from slightly below the soil line to an inch or more up the stem at the time the plant wilted. They consisted of dark-green water-soaked bands, which later dried brown. The upper line of demarcation between the killed and living tissue was distinct. The width of the girdling band on the stems aboveground was greater when the point of infection was farther from the soil line. The entire tops of young plants were usually invaded by the time the plant died, while the tops of older plants were not invaded by the fungus, probably because the woodiness of the stems apparently retarded the spread of the fungus. When one branch was invaded the fungus, almost without exception, advanced to the primary stem and eventually killed the entire top.

¹ Tucker, C. M. Taxonomy of the genus Phytophthora De Bary. Missouri Agr. Exp. Sta. Res. Bul. 153. 1931. (See pp. 100-101.)

² Leonian, L. H. Stem and fruit blight of peppers caused by *Phytophthora capsici* sp. nov. Phytopath. 12: 401-408. 1922.

On the leaves, the spots were at first small, circular or irregular in shape, and appeared scalded (Fig. 1). Later, they enlarged, became dried and bleached to a light-tan color, and were of a papery consistency. These areas eventually cracked and in some instances fell away from the unaffected tissue. No macroscopic evidence of the parasite was observed on the surface of affected leaf tissue. Entire leaves killed by these local lesions have not been observed, although extensive dead areas were common.

The fruit was infected through the stem ends by the advance of the fungus from stem lesions through the peduncle. Pod infections originating otherwise have not been observed. The peduncles were always discolored before macroscopical evidence of fruit invasion took place. The progress of the fungus through the fruit was determined by the dark-green,

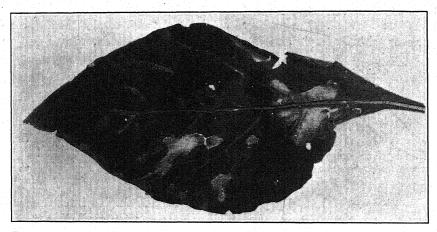


Fig. 1. Lesions on pepper leaf resulting from natural infection by *Phytophthora* capsici.

water-soaked appearance of the invaded cells in contrast to the light-green color of the uninvaded ones. The development of the fungous hyphae and fruiting structures on the surface of the pods lagged behind the advancing margin of disease from a few mm. to $1\frac{1}{2}$ cm. The short conidiophores grow out through the epidermis in compact clumps and produce the conidia. After several days the entire pepper pod was invaded and covered with fruiting structures of the fungus. Following this stage the pod dried out rapidly and remained attached to the plant in mummied form (Fig. 2).

CULTURE OF THE ORGANISM

The parasite was isolated from stems, leaves, and fruit. It did not produce sporangia abundantly in culture, but the oospores were plentiful. Cultures of the originally described *Phytophthora capsici* and of *P. terres*-

tria Sherb.³ were grown on various media simultaneously with the organism isolated from peppers in Florida. Always, the cultures from pepper were comparable and were distinct from *P. terrestria* Sherb.

INOCULATIONS

Uninjured pepper plants growing in the greenhouse and in field plots were inoculated by spraying with a water suspension of sporangia, and 54 per cent infection was obtained in the greenhouse and 19 per cent infection out of doors. All infected plants showed stem, branch, or leaf lesions.

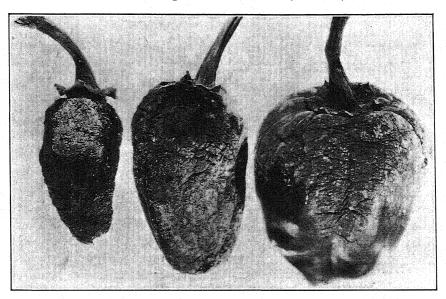


Fig. 2. Pepper pods showing 3 stages of the disease caused by *Phytophthora capsici* in the field. Most recently infected pod is on the right.

Other pepper plants growing in the greenhouse were inoculated with sections, 4 mm. square, of hard poured-agar media overgrown by the fungus mycelium. The inoculum was placed fungus side to the plant in the fork of the first branches, some of which had been injured previously. After 30 hours' incubation in a moist chamber the plants were placed on a greenhouse bench where the disease became evident 3 days after inoculation. Phytophthora capsici and the Phytophthora sp. isolated from peppers in Florida produced the disease in 100 per cent of the trials on both injured and uninjured pepper plants (Fig. 3). The check plants and those inoculated with P. terrestria did not become diseased.

³ Phytophthora terrestria Sherb. considered the same as P. parasitica Dastur, both having been considered as synonyms of P. omnivora De Bary. Leonian, L. H. Physiological studies on the genus Phytophthora. Amer. Jour. Bot. 12: 444-498. 1925.

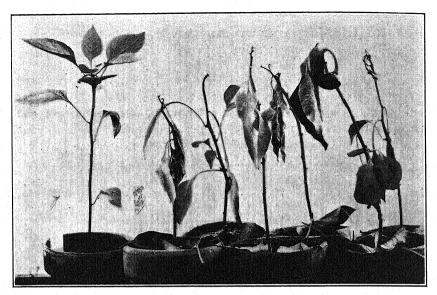


Fig. 3. Pepper plants inoculated with *Phytophthora capsici* from Florida. Check plant at the left.

Green pepper pods were surface-sterilized and inoculated in the laboratory and incubated in glass moist chambers. They were inoculated by placing on the sterilized and aseptically clipped end of the peduncle a small square of hard potato agar covered with the fungus, placing the mycelium side in contact with the plant tissue. Infection took place rapidly and the pods inoculated with the organisms from pepper developed similar disease symptoms that were indistinguishable from those on naturally infected pods. In both cases the organisms were reisolated, and they compared favorably with each other and the original cultures. The pods similarly inoculated with *P. terrestria* and the checks did not become diseased (Fig. 4).

DISCUSSION AND CONCLUSIONS

The description of the disease of peppers caused by *Phytophthora capsici* in New Mexico is not entirely comparable with the symptoms of the disease observed in Florida. Some variation may be accounted for because of different kinds or varieties of peppers, the symptoms developing in New Mexico being described on Chile peppers, while in Florida the disease appeared on Ruby King and California Wonder varieties. In Florida, the leaves were infected and portions of them were killed, whereas leaf infection was not mentioned in connection with the disease in New Mexico. In Florida, it was observed that all fruit infection occurred at the stem end

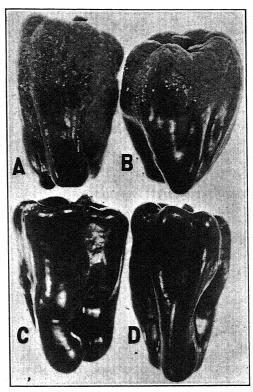


Fig. 4. Diseases of pepper pods resulting from artificial inoculations. A. *Phytophthora* capsici, from New Mexico. B. *P. capsici*, isolated from diseased plants in Florida. C. *P. terrestria*. D. Sterilized water (check).

as a result of the advance of the fungus from the branches through the peduncle. The fruit infection always advanced from the stem end to the blossom end of the pods, uniformly on all sides. The linear mode of advancement of the disease as described in New Mexico was not seen. In Florida, attacked pods always became completely overgrown by the parasite in contrast to frequent indefinite halts of advancement in the disease in New Mexico. The difference in symptoms may be accounted for largely by the differences in relative humidity during the respective growing seasons. Since these climatological data were not given in connection with the description of the disease in New Mexico, definite comparisons cannot be made. The relative humidity is generally high in Florida, especially in the Homestead section during the winter growing season. It may be that differences in relative humidity will account for the difference in the symptoms of the disease as described by Leonian for naturally infected

field plants and artificially inoculated greenhouse plants, this possibly replacing his suggested "mass-action" effect.

The fungus causing a blight of peppers in Florida was isolated and identified as *Phytophthora capsici*, previously described as causing a disease of Chile peppers in New Mexico. The diseases resulting from artificial inoculation of pepper plants with cultures of the New Mexico and Florida organisms were indistinguishable, whereas peppers inoculated at the same time with *P. terrestria* remained healthy.

This fungus, previously reported from continental United States but once, is herewith reported from Florida for the first time.

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SYNERGISM IN A BACTERIAL DISEASE OF HEDERA HELIX

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In the spring of 1931, several plants of the common, large-leaf type of English ivy, Hedera helix L., affected with a leaf spot were received by the Department of Plant Pathology at Cornell University for diagnosis. The plants were representative of a large shipment from Georgia consigned to a retail store in New York State. Microscopic examination revealed the presence of bacteria within the diseased tissues. Isolations were made from typical spots and, in certain instances, 2 distinct types of bacteria were obtained from a single lesion. Both of the organisms produced round, yellow colonies on beef-extract agar. They differed in that one type had a slower rate of growth and was somewhat lighter in color. Inoculation experiments demonstrated that the slower-growing organism (Type I) was pathogenic. The other organism, which grew more rapidly and was darker (Type II), proved to be nonpathogenic when used alone.

A bacterial disease of English ivy has been the subject of three different publications from Europe. In 1894, Lindau¹ described a disease of this plant and attributed its cause to a bacterium. He made no attempt, however, to culture the organism or prove its pathogenicity. Arnaud,² in 1920, reported what appeared to be the same disease from France and named the associated organism Bacterium hederae, n. sp. A description of the organism was omitted, however, and proof of its pathogenicity was again lacking. One year later, Killian³ obtained infection on ivy with a bacterium and reported the pathogen's reaction on several media. White⁴ first reported the presence of the disease in this country and in a later publication⁵ presented a complete description of the symptoms exhibited by affected plants together with considerable information in regard to various pathological phases of the problem.

From a comparison with the symptoms described in earlier reports, it was evident that we were dealing with the same disease. On the leaves, the first symptom of infection consists of a small water-soaked area about the

- ¹ Lindau, G. Der Epheukrebs. Ztschr. Pflanzenkr. 4: 1-3. 1894.
- ² Arnaud, G. Une maladie bactérienne du lierre (*Hedera helix* L.). Compt. Rend. Acad. Sci. (Paris) 171: 121-122. 1920.
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- ⁴ [White, R. P.] Bacterial leafspot of *Hedera helix*. New Jersey Agr. Exp. Sta. Nursery Dis. Notes 3 (6): 4. 1930. (Mimeographed.)
- ⁵ [White, R. P.] Diseases of *Hedera helix*. New Jersey Agr. Exp. Sta. Nursery Dis. Notes 4 (1): 1-4. 1931. (Mimeographed.)

point of entrance. As the spots increase in size, the lesions develop dark brown to black centers. In the final stages, and especially with mature leaves, the water-soaked margins around the spots disappear and the centers crack as they become dry. A distinct red purple margin is usually visible at this time. Natural infections on the leaf petioles and stems were not present on our material, but, according to White, such infections do occur.

In preliminary experiments to determine which of the 2 organisms was responsible for the disease, a series of plants was inoculated with Type I alone, another series with Type II alone, and, as a matter of interest, a third series with Types I and II together. The inoculations were made by placing the bacteria in small wounds made on the foliage by means of needle punctures. It was interesting to note that infection occurred first on those plants that had been inoculated with the mixture of Types I and II. The initial symptoms were visible in 6 days, and it was a week later before the plants inoculated with Type I alone showed evidences of infection. The wounded checks and the series inoculated with Type II alone gave negative results.

This experiment has been repeated 3 times with similar results. The disease acceleration obtained by mixing the 2 types of organisms can be shown by measurements of the diameters of spots on plants inoculated under similar conditions with either one or both of the two organisms. A set of these data are presented in the following table. Plants and leaves of the same relative age were used and the measurements were made 20 days after the plants had been inoculated.

Diameter	Number of spots								
of spots in mm.	Inoculum Type I	Inoculum Type II	Inoculum Types I and II	Wounded check					
0.0	0	54	1	54					
1.0	16	0	0	0					
1.5	13	0	0	0					
2.0	20	0	8	0					
2.5	2	0	16	0					
3.0	3	0	18	0					
3.5	0	0	0	0					
4.0	0	0	11	0					

In this particular experiment, the spots did not increase rapidly in size, due to cool temperatures prevailing in the greenhouse. It was very evident, however, that the bacteria representative of Type II had a distinct

⁶ See footnote 5.

accelerating action upon the pathogen, Type I. In other experiments, where the greenhouse temperatures have been higher, the difference in size of the spots was more striking.

It should be pointed out that the effect of the accelerator was not lasting. Approximately 4 weeks from the date of inoculation, the infections caused by Type I alone were similar in size and appearance to those resulting from Types I and II together. Furthermore, the addition of the accelerator at the time of inoculation did not have any significant effect on the relative percentages of infection. In all experiments thus far conducted, we have obtained practically 100 per cent infection with Type I alone and with Types I and II together, while all inoculations with the accelerator alone have given negative results.

Suggestions as to the exact nature of this case of synergism are not offered in this preliminary report, but the following observations are of interest. In 4 attempts to reisolate the pathogen and the accelerating organism from artificially produced lesions where both organisms had been used it was found that from the small spots the 2 organisms appeared in the plates in approximately equal numbers. From the large lesions the accelerating organism greatly predominated.

Since a complete description of the pathogen causing the leaf spot of ivy has never been published, the following work on its cultural characteristics was undertaken and is presented here. The specific name "hederae," as suggested by Arnaud, is used, although this investigator did not have the organism in pure culture. There seems to be no doubt, however, that he was working with the same disease, since his description of the symptoms is identical with ours. Furthermore, the name hederae has crept into literature dealing with phytopathology and, to avoid future confusion, should be retained. A complete description of the accelerating organism also is given, but without a name. It appears, however, to be similar to the epiphyte frequently referred to in European literature as Bacterium herbicola aureum. It should be stated that this accelerating organism is much more active biochemically than the pathogen.

Phytomonas hederae is a slender rod, Gram-negative and motile by means of a single polar flagellum. Spores are not formed. Its average size is 2.13 μ by 0.6 μ . On beef-extract-agar slants growth is good, filiform and amber yellow; its consistency is watery to butyrous; milk is cleared and finally becomes alkaline; gelatin is liquefied; nitrates are not reduced; indol and hydrogen sulphide are not formed; ammonia is produced in peptone broth; the following carbohydrates are fermented but with no production of gas, dextrose, levulose, galactose, xylose, lactose, sucrose, glycerol, and the sodium salts of acetic, citric, lactic, malic, and succinic

acid; and the following are not fermented, arabinose, rhamnose, maltose, salicin, starch, cellulose, and the sodium salt of formic acid. The organism is a faculative anaerobe.

The accelerating organism is a short rod, at times oval with a central granule, Gram-negative and motile by means of one to two polar flagella. There are no spores. Its average size is $1.7\,\mu$ by $0.9\,\mu$. On beef-extractagar slants growth is good, filiform, and antimony yellow; its consistency is butyrous; milk at first turns alkaline but later becomes neutral or slightly acid; nitrates are reduced; indol and hydrogen sulphide are not formed; ammonia is produced in peptone broth; the following carbohydrates are fermented rapidly without gas, dextrose, levulose, galactose, arabinose, xylose, rhamnose, maltose, sucrose, glycerol, mannitol, salicin, and the sodium salts of acetic, citric, lactic, malic, and succinic acids; and the following are not fermented, lactose, starch, cellulose, and the sodium salts of formic and tartaric acid. The organism is a definite facultative anaerobe.

Further work on pathological phases of the problem is in progress.

SUMMARY

A case of synergism in a bacterial disease of English ivy is described. From certain leaves, 2 distinct types of bacteria were isolated. The organisms differed slightly in color and rate of growth on beef-extract agar. The slower-growing bacterium proved to be the pathogen, while the other organism was nonpathonegic when used alone. When the 2 organisms were combined for the purpose of inoculation, the nonpathogen was shown to have a distinct accelerating action upon the disease complex.

A complete description of the pathogen, *Phytomonas hederae*, is given. The accelerating organism also is described, but without a name.

DEPARTMENT OF PLANT PATHOLOGY, CORNELL UNIVERSITY, ITHACA, N. Y.

PHYTOPATHOLOGICAL NOTES

Bacterial and Fungous Flora in Certain Sulphur Fungicides.—In conducting certain toxicity tests with various sulphur fungicides, there was experienced a degree of contamination on seeded plates that could not be explained through any procedure of conventional technique. of this situation, attention was given to the possible contamination that certain sulphur fungicides might be carrying. Representative samples of 14 various sulphur fungicides were collected that included, for the most part, mixtures of straight elemental sulphur, modified sulphurs, which, in addition to the elemental sulphur, may contain hydrated lime and wettable agents, and suspended sulphurs. Qualitative procedure, such as the inoculation of a tube of bouillon with a small loopful of the fungicide, followed by a 5-day incubation period and then seeding on agar plates will provide evidence in contaminations of dominant organisms. In order to determine the probable contaminations from a quantitative standpoint, the technique used for soil-flora studies was adopted. This procedure included the use of 1 gm. of the fungicide with 100 cc. of sterile water in a sterile This mixture (A) was placed on a shaker for 30 minutes and additional dilutions of 10⁻⁴ (B) and 10⁻⁵ (C) were made from the original mixture. From each of these 3 dilutions, 1 cc. was withdrawn from A and 1/10 cc. from B and C with a sterile pipette and seeded with melted agar media in sterile plates. Only 3 types of media were selected for this work, namely, nutrient agar, nutrient glucose agar, and Ashby's agar. It was found that these media were favorable for growth of certain contaminating organisms but would not represent a range of media favorable for isolating all possible contaminating organisms, especially in those sulphur fungicides that are modified with casein and other wettable agents. Some of the fungicidal mixtures showed bacteria present in numbers exceding 1,000,000 per gm., while fungi were found in numbers up to 30,000 per gm. These results strongly indicate that the contaminations are much higher than would normally be expected for natural air-exposed materials.

The bacterial flora for the most part represented species of the *Bacterium fluorescens* group, and the fungi were species of Aspergillus and Penicillium. No study was made as to the possible presence of anaerobic or gas-producing forms of bacteria. That contaminations should be encountered is natural when one considers the various steps in the preparation and handling for retail trade of various forms of dry sulphur fungicides. Particularly in those types of modified sulphurs where caseinate and similar substitutes are added to improve the wetting and physical properties there is found a greater contamination than is met with in samples of elemental sulphur. Samples of modified sulphurs 2 years of age showed a

heavier contaminating flora than did fresh samples. Suspended sulphurs, especially from by-products in gas manufacturing, showed the heaviest bacterial flora, with over 1,000,000 per gm. of material. When the original dilutions of the fungicides were allowed to stand 4 weeks and then seeded, the number of contaminating organisms invariably increased. No attempt was made to determine whether the organisms found were either beneficial or detrimental to the efficiency of the fungicide.

These facts are not presented as the result of a complete investigation of the subject but principally for the purpose of raising 3 questions. (1) Is a contaminating flora in certain sulphur fungicides beneficial or detrimental? (2) How much contamination should be permitted? (3) Whether it is not pertinent, in view of facts reported, that precaution should be taken in determining the purity of certain sulphur fungicides before using them for toxicity tests or determining their fungicidal or bactericidal action.—

J. F. Adams, Agricultural Experiment Station, University of Delaware, Newark, Delaware.

Notes on Phyllosticta rabiei on Chick Pea, II.—Some years after Trotter¹ published the results of his excellent work on the fungus Phyllosticta rabiei (Pass.) Trotter, Labrousse,² and more recently the writer,³ published partial confirmation of Trotter's studies. The writer pointed out that only a small percentage (less than 5 per cent) of the conidia were septate, and those only faintly. While the writer agreed with Trotter that the fungus was closer to Phyllosticta than to Ascochyta, it was pointed out by him that the genetic connection with Ascochyta was possibly closer than Trotter suspected. It must be borne in mind, however, that nonseptate conidia very frequently form cross walls just previous to germination.

Recently, Labrousse carried this study still further and proposed Ascochyta rabiei (Pass.) nov. comb. He pointed out that A. pinodella L. K. Jones has a high percentage of nonseptate spores and that he was in agreement with Trotter and the writer that the chick-pea fungus is somewhere genetically connected with the leguminous Ascochytae.

The difficulty of placing the border-line fungi in some of these genera of Fungi Imperfecti is well known and the Phyllosticta-Ascochyta-Stagonospora complex is one of the most troublesome. While the writer realizes

¹ Trotter, A. La "rabbia" o "anthracnosi" del cece ed il suo produttore. Riv. Patal. Veg., n.s. 9: 105-114. 1918.

² Labrousse, L. L'anthracnose du pois-chiche (*Cicer arietinum*). Rev. Path. Vég. et Ent. Agr. 17: 174-177. 1930.

³ Sprague, R. Notes on *Phyllosticta rabiei* on chick pea. Phytopath. 20: 591-593. 1930.

⁴ Labrousse, F. L'anthracnose du pois-chiche (2° note). Rev. Path. Vég. et Ent. Agr. 18: 226-231. 1931.

that technically Labrousse's position is well open to support, he feels that Trotter's diagnosis is the more conservatively taken. If we are to place every Phyllosticta with a small percentage of septate spores in the genus Ascochyta it will be necessary, for the sake of consistency, to place some physiologic forms of Ascochyta pisi Lib. in the genus Stagonospora or, at least, in the doubtful genus Stagonosporopsis Died. We would then have the awkward situation of a certain physiologic form of a fungus or even a growth form recognized as a full-fledged species in an entirely different genus.

As to the appropriateness of the genus Phyllosticta for species that attack other organs in addition to leaves, it may be noted that such fungi as *Phyllosticta solitaria* Ell. and Ev., which attacks twigs and fruits as well as leaves, have long stood undisturbed in the genus Phyllosticta. In an herbaceous plant, such as the chick pea, there is even less reason for attaching any taxonomic value to leaf versus stem parts.

The writer does not consider Ascochyta pinodella L. K. Jones as a parallel case, because, very frequently, collections of this fungus show close to 100 per cent of the spores septate, while in mature specimens of Phyllosticta rabiei, 96 to 98 per cent of the spores are nonseptate. That is, A. pinodella is typically uniseptate, while P. rabiei is predominatingly nonseptate. The routine worker should have no particular difficulty in placing a collection of A. pinodella in its accepted niche, but it would require considerable study for him to see any connection of Phyllosticta rabiei with Ascochyta. In order to avoid further confusion in this the writer pleads that Trotter's combination of P. rabiei (Pass.) Trotter be retained.—Roderick Sprague, United States Department of Agriculture, at Oregon State Agricultural College, Corvallis, Oregon.

Color Variations in Bacterial Plant Pathogens.—In 1930 the writer published a note on a white strain of Aplanobacter michiganense E. F. Sm., the cause of bacterial canker of tomato. Since that time white variants have been observed in three other yellow bacterial pathogens, namely, Bacterium campestre (Pam.) E. F. Sm., Bact. vesicatorium Doidge, and Bact. cucurbitae Bryan. In all three cases, the variation was discovered in old beef-agar stock cultures, in the form of wedges in otherwise normally yellow surface growth. In the case of Bact. vesicatorium the white strain was also isolated directly from typically spotted fruit from Mexico in 1931 and proved to be as infectious as the yellow strain, producing typical spots on fruits and leaves. The variations in cultures have occurred only in an occasional tube. Cultures of sister colonies of the same age and held under identical conditions remained normal.

In the case of each of the three pathogens mentioned above, the white strain was separated by means of poured plates. Transfers from single white colonies were replated, giving only white colonies. Transfers from these colonies were then used for inoculation of suitable hosts in the hothouse. The white Bacterium campestre was as virulent as the yellow strain from the same culture, i.e., the one from which the white was separated. Bacterium vesicatorium was also as infectious as the normal strain, but in the case of Bact. cucurbitae the white strain was decidedly less virulent than the yellow one.

The white color, in the case of all these bacteria, persists in the various culture media and comes out pure when reisolations are made from inoculated plants. Reversion to yellow has occurred in only an occasional culture in stocks held for months in the ice box under conditions similar to those under which the original variation appeared.

The strains of each organism have been compared in the most common culture media: beef agar and broth, beef gelatin, potato cylinders, litmus milk, and nitrate broth, without discovering any differences aside from color.

It seems probable that other yellow pathogens, in both nature and in culture, produce color variants that have not been recognized as such. Since Aplanobacter michiganense also has a pink strain, this color as well as the white should be borne in mind when working with "normally" yellow organisms.—Mary K. Bryan, Department of Agriculture, Washington, D. C.

A New Form of Oat Stem Rust from a Barberry Area.—A new physiologic form of oat stem rust (Puccinia graminis avenae) was identified in two collections made in 1930 in a barberry area near Jefferson, Wisconsin. Many barberry bushes have been found in this area in the past, and at the time the collections were made there were barberries in the vicinity, over 4,000 bushes and seedlings being found in the county in 1930. Inasmuch as oat fields near the bushes were rusted and a general infection had not yet appeared in the fields of surrounding territory, it seems logical to assume that the new form came from near-by barberries. The new form, which has been called "form 10," has not yet been found elsewhere. Unsuccessful attempts were made to isolate it again from Jefferson County collections in 1931.

The two collections of form 10 were cultured in the greenhouse for more than 8 months and both have behaved consistently on the differential varieties of oats. Form 10 attacks Richland (C. I. 787), which is resistant to forms 1, 3, and 7, also forms 2 and 5, the most prevalent forms of oat stem rust in the United States. While form 4 and 6 also infect Richland

heavily, White Tartar (C. I. 551) is very susceptible to these forms but resistant to form 10; and Joanette Strain (C. I. 2660), resistant to form 4 and susceptible to form 6, reacts indeterminately to form 10 (infection type X), the reaction resembling that of this variety to form 5 as described by Stakman, Levine, and Bailey.¹

Since it has been shown experimentally that new forms of wheat stem rust are produced by hybridization on the barberry and since there is evidence that they actually are being produced in nature, it appears probable that new forms of oat stem rust may originate in the same way. All the evidence points to the fact that form 10 probably originated through hybridization or segregation on barberries, but there is a question whether it has become established. (Cooperative investigations between the Division of Barberry Eradication and the Division of Cereal Crops and Diseases, United States Department of Agriculture, and the Minnesota Agricultural Experiment Station.)—RALPH U. COTTER, University Farm, St. Paul, Minn.

¹ Stakman, E. C., M. N. Levine, and D. L. Bailey. Biologic forms of *Puccinia graminis* on varieties of *Avena* spp. Jour. Agr. Res. 24: 1013-1018. 1923.

² Stakman, E. C., Lee Hines, Ralph U. Cotter, and M. N. Levine. Physiologic forms of *Puccinia graminis* produced on barberries in nature. (Abst.) Phytopath. 22: 25. 1932.

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A STUDY OF TWO SEPTORIA LEAF SPOTS OF CELERY¹

L. C. COCHEAN (Accepted for publication February 29, 1932.)

INTRODUCTION

Late blight is one of the most serious diseases of celery in the United States. During a preliminary study of the disease in the fall of 1928, it was pointed out to the writer by Ray Nelson of the Michigan Agricultural Experiment Station that he had had under observation for 8 to 10 years 2 distinctly different types of spots caused by late blight on celery. Upon further examination, it was found that there are 2 distinct forms of the disease caused by 2 distinct species of the genus Septoria. It is the purpose of this paper to present studies that will help to distinguish these fungi and to clarify nomenclature and life histories.

HISTORY OF LATE BLIGHT

According to Chupp (7, p. 89), late blight of celery is probably about as old as the cultivation of celery and may have been observed as early as 1840. The first published report of it as a distinct disease was not made until 1890, when it was discovered in Italy by Briosi and Cavara. The disease was first recognized in the United States by Chester (4)(5) in Delaware in 1891. Rostrup (16) identified the disease in Denmark in 1893. Prillieux and Delacroix (22) found the disease in France in 1894, and Sorauer (26) in Germany in 1896. Pethybridge (21) and Chittenden (6) reported the disease as serious in all parts of the British Isles in 1910.

Shortly after Chester found Septoria on celery in Delaware, it was reported from almost all the important celery-growing regions in the United States. Duggar and Bailey (12) reported it as the cause of enormous losses in central and western New York in 1897. Rogers (23) accredited the disease with serious losses in California in 1897. Extreme losses occurred in California-shipped celery during the winter of 1908, when 1,950 cars, valued

¹ Journal article 84 (n.s.) from the Agricultural Experiment Station of Michigan State College, published by permission of the Director.

This paper includes material presented by the writer as a thesis in partial fulfillment of the requirements for the M.S. degree, Department of Botany, Michigan State College, June, 1930.

The writer wishes to express his indebtedness to Dr. E. A. Bessey, under whose direction this work was done, for correcting the manuscript and for invaluable advice and suggestions. He also is indebted to Dr. Ray Nelson for many suggestions throughout the work and for help with the photographic work.

at \$550,000 were lost. Coons and Levin (9) and Coons (8) reported a loss estimated at over \$1,000,000 for Michigan alone in 1915. In a survey of market diseases, Link and Gardner (19) found heavy losses in shipped celery and celeriac in 1918.

Since the first published note upon late blight, its occurrence has been reported until at the present time Chupp (7) and Owens (20), in their textbooks, state that it is the most cosmopolitan of all celery diseases. Thus in 40 years, the disease has become the most serious malady of celery.

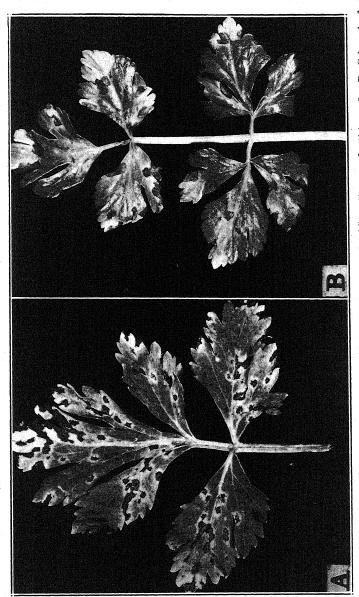
A study of the literature showed that Dorogin (11), in 1915, had observed two organisms causing two distinct types of the disease in Russia. He identified one with the typical form reported from other regions, but considered the other a new species and gave it a new name. Foster and Weber (14) described two sizes of leaf spots caused by Septoria on celery in Florida in 1924, but they agreed with Laibach (18) and regarded these as strains of the organism described by Briosi and Cavara and not as separate species.

Aside from these reports and the observations of Nelson, mentioned above, only one species of Septoria is usually listed as a parasite on celery. (Anderson (2), Seymour (25)). Since the writer wishes to present studies that will differentiate the two forms before discussing the question of names, temporary names or symptom names will be used to designate the forms in this paper. One of the fungi produces a larger leaf lesion than the other and will be designated as the large-spot form and the other the small-spot form.

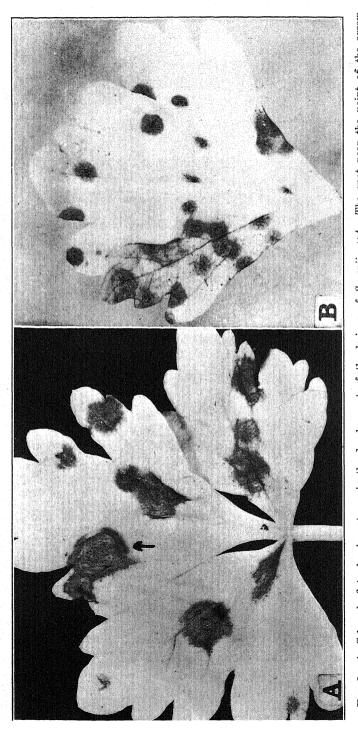
SYMPTOMS

Under optimum conditions, a period of from 9 to 12 days is necessary for these fungi to enter the host and establish themselves to the extent of the production of visible lesions. Under adverse conditions of temperature, moisture, or health of the host, this period may be lengthened but is never shortened. Early symptoms of infection caused by the 2 fungi are very similar, but, as they develop, the lesions soon become easily distinguishable.

The large-spot form (Fig. 1, B and Fig. 2, A) usually requires an incubation period of a day or 2 less than that of the small-spot form. The first visible evidence of infection is in the form of a small chlorotic fleck. The first definite lesion is marked by collapse of the host tissue containing and adjacent to the fungus. It seems that the fungus may at a given state in its development secrete some toxic substance that actually kills the host cells for some distance, and this is followed by collapse of tissue, even when the hyphae are not present. This type of lesion in its early stages is very similar in appearance to that caused by *Cercospora apiicola*. In general, the spots are from 3 to 6 mm. in diameter. If the atmosphere is very moist, small, black, scattered pycnidia may become evident in the center of the spot 2 or 3 days after the first signs of collapse. These pycnidia are never



B. Celery leaf Fig. 1. A. Celery leaf infected with small-spot form of Septovia (8. apii graveolentis). $\times 2$. infected with large-spot form of Septoria (S. apii). × 3.



Frg. 2. A. Celery leaflet, showing stages in the development of the lesions of S. apii. ×4. The spot near the point of the arrow ows scattered pyenidia in the center. B. Celery leaflet, showing stages in the development of the lesions of S. apii graveolentis. ×4. ome of the spots show pyenidia in uncollapsed tissue. In all cases the pyenidia are clustered and many are actually fused.

found along the margin of the spot and are scattered singly, never clustered. If the weather continues cool and moist, the spot may increase to 2 or 3 times its original size. If but a single lesion is present on a leaf, the leaf may not show much change for a week, but 2 or 3 lesions are sufficient to cause yellowing in as many days.

The small-spot type (Fig. 1, A and Fig. 2, B) differs from the large in that as soon as, or sometimes even before, chlorotic spots appear on the leaves fruiting bodies may be found. Pycnidia may be found outside of the indefinite margin of the spot in apparently healthy tissue. The killing and collapse of the tissue start at the middle of the spot and progress outward. The pycnidia that were buried in the leaf parenchyma are now exposed as erumpent black bodies closely crowded together and often fused together in twos and threes. In general, the spot is from less than ½ mm. to 2 mm. in diameter and never attains the size of the large-spot type. The easy-blanching varieties of celery are able to endure from 3 to 5 of these spots. The green celeries seem to be able to tolerate numerous spots before yellowing occurs. If the infection is severe in damp weather, the spots may fuse, giving a brownish black appearance, and the leaf rots with a soft, wet root without yellowing.

The striking differences between the 2 types of spots are, thus, the size and the time and manner of production of fruiting bodies. The mature or completely developed spot of the large-spot type is definite in outline, brown to reddish brown in color, and surrounded by a darker reddish brown border, with scattered pycnidia near the center. The small-type spot is indefinite in outline, brownish to black or sometimes gray, with black pycnidia that may be found both in the necrotic portion of the spot and often in the green tissue surrounding it. The pycnidia in the spot are erumpent, closely crowded, and some are fused in twos and threes. Although the difference in size of the spots of the 2 types is quite striking, a comparison can only be relative, since spots produced by either of the fungi may increase in size for several weeks or even until the host is entirely killed. Comparing the 2 under similar conditions, the lesion produced by the smallspot form on leaves ranges from ½ to 3½ mm. in diameter, and the large type may attain a diameter from $1\frac{1}{2}$ to 10 mm. In the field, the small-spot type is usually $\frac{1}{3}$ to $\frac{1}{2}$ the diameter of the large one.

ETIOLOGY

Both Septorias were collected on celery from the Kalamazoo marshes, and isolations from these were used in cultural wark. Single-spore cultures were made by the dilution-plate method. After 2 weeks of incubation, spore suspensions of each form were made, and 5 healthy plants in separate infection chambers were atomized with each. The plants were allowed to remain in the propagating cases for 48 hours, when they were removed to the green-

house bench. After 13 days both lots of plants showed typical types of the disease, while the checks remained entirely healthy. Isolations from lesions gave characteristic growth on potato-dextrose agar for both forms of the fungus. In the course of this study pure-culture inoculations were repeated many times.

To avoid individual differences in celery plants, which might be argued as a cause of the difference in size of spot caused by the two fungi, the two halves of a single plant were atomized with spore suspensions of the two forms, respectively. After two weeks the characteristic types of spots were apparent on their respective sides.

MORPHOLOGY OF THE TWO SPECIES OF SEPTORIA AND THEIR RELATION TO HOST TISSUE

The mycelia of these two forms of Septoria in sections of diseased leaves are very similar and are composed of twisted and interwoven, irregularly septate, brownish to black, irregularly thickened hyphae. At the corners of the host cells, where the mycelium is intercellular, there may be whole fascicles of hyphae producing knot and bulb-like formations of irregular outline. The mycelium of the small-spot form is from 1.5 to $4.5\,\mu$ in diameter, while that of the large-spot form is 1.0 to $5.5\,\mu$ in diameter.

In sections through lesions the mycelium of the small-spot form is found to be much more extensive than that of the large one. Strands of mycelium may be found as far as 1,500 μ beyond the edge of the collapsed tissue. Absence of the mycelium in portions of the lesions of large-spot type points to the possible production of a toxic substance that kills the tissues in advance of the fungus and helps to explain the shorter incubation period and the increased size of the spot produced by the large-spot form.

The pycnidia of the 2 forms differ in size and shape and in their time and place of appearance. The pycnidia of the small-spot form are visible very early and are one of the first signs that distinguish a lesion, while the pycnidia of the large-spot form are seldom evident on well-aerated leaves and, when present, occur only in the central portion of the lesion. Pycnidia of the small-spot form range from 73 to $147~\mu^2$ in diameter, with ostioles of from 1/3 to 1/2 the diameter of the pycnidia. The pycnidia of the large-spot form are smaller, ranging from 65 to 95 μ in diameter, with ostioles of less than 1/4 the diameter of the pycnidia. The pycnidia of the large-spot form are practically spherical and are always borne singly unless in very old lesions that have been in a moist atmosphere, but those of the small-spot form are usually crowded and even fused.

The spores (Fig. 3) or conidia of both types are long, slender, filiform, hyaline, faintly septate bodies. The spores of the 2 fungi differ markedly

² The measurements were made on pycnidia on leaves that had been cleared in carbol-turpentine.

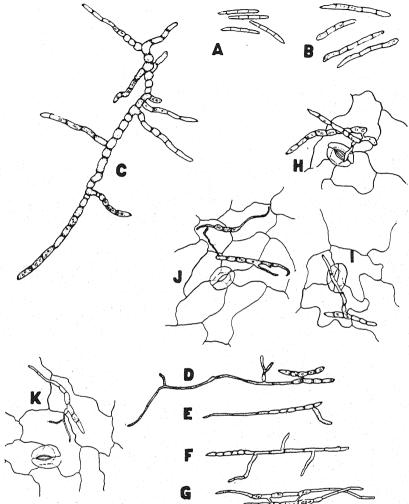


Fig. 3. Camera-lucida drawings of spores. A. Spores of the large-spot type Septoria (Septoria apii). B. Spores of the small-spot type Septoria (S. apii graveolentis). C. 3-day-old germinating spore of the small-spot type. D. and G. Germinating spores that have fused. E. and F. Germinating spores of large-spot type. H. Germinating spores with germ tube passing over a stoma. I. Germ tube passing directly over a stoma J. Fused spores on epidermis. K. Germinating spore with germ tube entering a cell through the entielmal wall.

in size, shape, and appearance. The spores of the small-spot form (Fig. 3, B) are slightly flexuose, blunt at the ends, granulose, with several indistinct septa, and vary from 22.5 to $58.5~\mu$ in length by 1.5 to $3.0~\mu$ in diameter. The mean for length was $35.0~\mu$ and $2.2~\mu$ for diameter. In plotting a curve of the measurements of the spores, it was found that 92 per cent would be included in lengths between 28.5 and $50.2~\mu$ and 66 per cent between 31.5

and $42.7 \,\mu$. The number of septa varied from 0 to 7. Ten per cent had less than 3 septa, 60 per cent had 3 septa, 18 per cent 4 septa, 10 per cent 5 septa, and 2 per cent had more than 5 septa.

The spores (Fig. 3, A) of the large-spot form were straight or slightly curved, minutely granular, with indistinct septa. In size the spores varied from 13.5 to 34.2 μ in length and from 1 to $2\frac{1}{2}\,\mu$ in diameter. The mean was 24.6 μ for length and 1.8 μ for diameter. In plotting a curve as before, it was found that 86 per cent of the spore lengths were between 21 and 28.5 μ . The number of septa varied from 0 to 4. One and one-half per cent had less than 2 septa, 34 per cent had 2 septa, 64 per cent had 3 septa, and $\frac{1}{2}$ of 1 per cent had 4 septa.

The above measurements were made from a fresh spore suspension taken from spots of several plants and prepared in the following way: Small sections of diseased spots on leaves from isolated plants that had been infected with pure cultures of the organism were placed in a test-tube containing sterile water. The tube was agitated so that the mature spores were washed from the cirri on the ostioles of the pycnidia. The suspension was placed on a slide and the spores were measured under oil immersion. Septa of fresh spores were very indistinct, so the septation counts were made on spores that had been fixed and stained with gentian violet. One thousand spores of each type were measured.

SEARCH FOR PERFECT STAGE

Stevens (27) and Gäumann (15) report several Septoria species other than those on celery, which in their perfect stage are members of the genera Leptosphaeria and Mycosphaerella. Klebahn (16) found a Pleospora on overwintered celery leaves but failed to get infection when he sprayed the ascospores on healthy celery leaves. The writer observed perithecia of Leptosphaeria and Ophiobolus in old Septoria lesions on overwintered celery leaf stalks, but single ascospore cultures on potato agar did not produce growth characteristic of either Septoria species.

Stevens (27) reported success with ultra-violet light in inducing the perfect stage of certain Ascomycetes. The writer exposed single-spore culcultures and single-spore-mated cultures of the two types of Septoria in open Petri dishes at 50 cm. from a mercury vapor lamp, but no evidence of an ascigerous stage was observed. At exposures above 20 seconds the fungi were either killed or inactivated so that their growth was greatly reduced.

Cultures of both of the fungi on standard laboratory agars along with cultures on steamed celery stems and leaves were stored at 2-3° C. for a period of 16 weeks. The cultures were examined from time to time, but a perfect stage was not found, although there appeared sclerotium-like mats in the bottom of the tubes of the steamed celery-leaf and leaf-stalk cultures of both types.

CULTURAL CHARACTERISTICS

In pure culture both fungi were found to grow well on many of the common laboratory media. The following media were used: potato-dextrose agar, prune agar, Coons' synthetic agar, hard oat agar, corn-meal agar, nutrient dextrose agar, celery-leaf-stalk-decoction agar, horse-dung-decoction agar, steamed celery-leaf stalks, carrot plugs, sterile filter paper, steamed water-cress stems, steamed parsley leaves, and steamed muck. The amount of growth on the various media varied in proportion to the amount of nutrient afforded the fungi, but the type of growth produced by the two fungi was quite markedly different (Fig. 4). The large-spot form produced

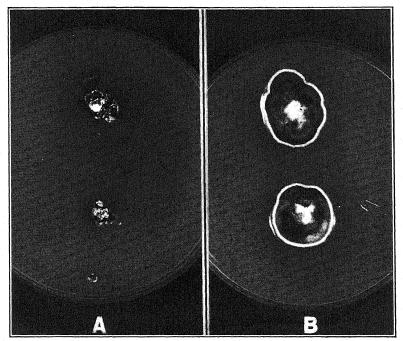


Fig. 4. Fifteen-day-old cultures on potato-dextrose agar. A. Septoria apii graveolentis. B. S. apii.

a colony of nearly twice the size of that of the small-spot form under the same conditions, and the growth was of a more spreading type, with fewer fruiting bodies. The small-spot form produced a more tufted growth of somewhat cheesy consistency that, upon examination, proved to be masses of pycnidia. Both fungi produced brownish black submerged mycelia and white aerial mycelia. The submerged mycelium of the small-spot form was quite ropy as compared to that of the large form. On potato-dextrose agar the large-spot organism produced a purple region in the medium around the colony, but the small-spot form produced no color.

Neither of the fungi was able to grow on sterile muck, and only a very scant growth occurred on corn-meal agar. The large-spot form grew luxuriantly on all the other media except the celery-decoction agar, where the growth was very scant and no fruiting bodies were formed. On this same agar the small-spot form grew and produced considerable mycelium with clumps of pycnidia. In a later experiment it was noticed that the large-spot form did not infect celery-leaf stalks. Since the base infusion of the celery-decoction agar was made by steaming celery-leaf stalks in water, it would seem that the growth of the fungus on agar might be correlated with that on the celery plant. However, both fungi grew equally well when they were placed on whole celery-leaf stalks that had been steamed.

THERMAL DEATH POINTS OF SPORES

As a further point of distinction it was found that the spores of the 2 fungi differed in their ability to withstand heat. Suspensions of mature spores in capillary tubes were exposed to a range of temperatures in water baths for a period of 10 minutes and then plated in potato agar. The results are recorded in table 1.

TABLE 1.—Therma	death	point of	spores, 10	minutes	in	capillary	tubes
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Temperature	40° C.	41° C.	42° C.	43° C.	44° C.	45 °C.	Check
Small-spot form	+ - a	+		_	-	-	+++
Large-spot form	+++a	+++	+++	++	-	-	+++

² +++ indicates numerous colonies developing, + indicates only a few, and - indicates no growth at all.

VEGETATIVE GROWTH AT VARIOUS TEMPERATURES

The season in which the diseases appear in the field, together with their reactions to controlled conditions in the greenhouse, suggests a rather sharp susceptibility on the part of the parasites to high temperatures. Thomas and Muller (29) and Campanile (10) found better growth of the celery Septoria at from 13° to 19° C. than at from 22° to 25° C. Owens (20) says that the disease caused by Septoria on celery is a cool-weather disease, the fungus thriving best between 60° and 70° F. To obtain further information on the temperature relations of the fungi, use was made of a modification of a Ganong differential thermostat. This piece of apparatus consists of low- and high-temperature chambers, between which are 12 small compartments that range in temperature from that of the low-temperature chamber to that of the high one.

Plantings of both types of the celery Septorias were placed on respective halves of Petri dishes on potato-dextrose agar and 3 dishes placed in each comprehent. The average temperature and growth after 18 days are recorded in table 2.

The 2 fungi are shown in table 2 to be distinctly different in their temperature requirement for growth. The large-spot type grows at tem-

TABLE 2.—Growth of celery Septorias (on potato-dextrose agar) after 18 days at various temperatures

Compart-	Tempera-	Large-s _l	oot form	Small-spot form					
ment number	ture range ° C.	Av. diam. of colony in mm.	Character of growth	Av. diam. of colony in mm.	Character of growth				
1	9–12	9–12 7.0		none	tufted				
			growth						
2	11½-15	7.5		very					
				slight	small white				
3	17-19	18.0	good	6.5	aerial mycelium				
			growth		good growth				
4	20-21	21.0	"	6.0	"				
5	22-23	26.0	luxuriant	6.5					
			growth						
6	23-24	26.0	very	5.0	"				
			black						
7	24-25	23.0	no aerial	slight	small and				
			growth		black				
8	26-27	13.0		none					
9	27-28	only a few		"					
		filaments			and the second				
10	$28\frac{1}{2} - 30$	none		"					
11	31-34	"		"					
12	38-42								
-									

peratures between 10° and 27° C. with an optimum of between 22° to 24° C. The small type grows at temperatures between 14° and 25° C. with an optimum between 18° to 22° C. The inhibition of growth at low temperatures points to the possibility of control of the small-spot type, which causes serious damage to celery in storage, by lowering the temperature of the storage chamber to a point at which the fungus will not grow.

EFFECT OF THE REACTION OF THE MEDIUM

Both of the celery Septorias grew well on a wide variety of media, which also varied widely in hydrogen-ion concentration. To test the reaction of the fungi to differences in pH, a series of media was prepared in the following manner. Since it was impossible to harden agar in solutions of high acidity, a liquid medium was used. The ordinary base of Coons' synthetic medium was used and sterile N/10 NaOH or HCl was added to make a variable series as determined by the colorimetric method. The reaction of the medium varied from pH 3.0 to 8.8 by 0.2 point intervals. Thirty cubic centimeters of the base solution were placed in 75 cc. Erlen-

meyer flasks in which small filter-paper cones had been placed, and the flasks were autoclaved. The culture flasks were inoculated by placing on the tip of the cone a small piece of agar containing mycelium. Both fungi grew well throughout the series. The large-spot type grew slightly more luxuriantly on the acid side of the scale, but the small-spot type showed no specificity. Campanile (10), working with what probably is the large-spot fungus of this study, reported more spread of the fungus on neutral or slightly alkaline media.

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SPORE GERMINATION AT DIFFERENT TEMPERATURES

A suspension of mature spores in tap water was obtained from leaves and placed in hanging drops on the under sides of glass slides that had been rimmed with vaseline. The slides were placed on supports in Petri dishes that had been lined with moist filter paper and the dishes were placed in the compartments of the Ganong thermostat. The slides were allowed to remain exposed to the different temperatures for a period of 14 hours, when they were removed and the germination stopped in the following manner. The slides were placed on a rack over an open dish of formal-dehyde under a bell jar. The germinating spores were soon killed and the average lengths of the germ tubes, as based on measurements of 50 spores from each lot, are shown in table 3.

The results of these germination tests agree fairly well in temperature range with those of vegetative growth. The only fact worthy of note is that a markedly smaller percentage of spores of the large-spot form

TABLE 3.—Germination of spores of celery Septorias at different temperatures for 14 hours

	Large-sp	ot form	Small-spot form						
Temperature (° C.)	Av. length of germ tube in μ	Germination	Av. length of germ tube in μ	Germination					
¥		Per cent		Per cent					
9 –12	3.1	14	10.1	82					
$11\frac{1}{2}-15$	2.8	18	11.2	90					
17 –19	6.9	26	23.7	94					
20 -21	6.9	30	41.6	92					
22 -23	11.1	28	43.3	94					
23 -24	17.3	40	42.3	88					
$24 - 25\frac{1}{2}$	16.2	38	45.5	90					
26 –27	14.7	26	27.6	60					
27 –28	6.5	24	9.1	20					
$28\frac{1}{2} - 30$	1.0	12							
31 –34		Act of the							
38 -42									

germinated, and the germ tubes were much shorter, indicating a longer incubation period. Campanile (10) did not get spore germination in sterile distilled water. It is quite possible that she worked with the large-spot form.

OXYGEN RELATION TO SPORE GERMINATION

Four capillary tubes were filled with a suspension of Septoria spores and sealed at each end, so that the liquid entirely filled the cavity of the tubes. Two other capillary tubes were filled and left open at each end. The tubes were placed in a culture room at 20–21°C. for 4 days. When the tubes were examined, there was no germination in the closed tubes, but the open tubes showed germination near the open ends.

OVERWINTERING STUDIES

The increased possibility of wide dissemination and early initial infection of both types of late blight makes the question of saprophytic growth on field soil very important. Two sets of 6 test-tubes each of sterile muck were inoculated by placing a single drop of a heavy spore suspension of each of the respective Septorias on the surface of the muck. In 2 more sets of 6 tubes, inoculations were made by transferring a small piece of agar containing mycelium. No growth, either macroscopic or microscopic, was observed in the tubes inoculated with the spore suspension. Only a few small filaments were found in the tubes in which the agar transfers were made, and these probably grew from the agar, as the soil did not support growth.

During the first week of April, 1930, partly decayed celery leaves from the previous year's crop, with definite lesions on them, were collected near Kalamazoo and Decatur, Michigan. The leaves examined in the laboratory revealed the presence of new and old pycnidia. A spore suspension obtained from the spots was sprayed on healthy celery plants and 14 days later the plants were badly affected with disease of the large-spot type. A similar collection, made in January of 1932, showed the presence of viable spores of the small-spot form on overwintered celery leaves.

LONGEVITY OF SPORES

The actual age at which Septoria spores lose their viability is not definitely known. Their longevity depends largely on storage conditions. Coons and Levin (9) recommend the use of 2- to 3-year-old celery seed in Michigan. Krout (17) found that conidia on celery leaves and on celery seed were dead in 8 to 11 months, and conidia in pycnidia on seed and pedicels failed to germinate after aging for two to three years. Flachs (13) reported the spores in pycnidia viable for one to two years. It was impossible for the writer to make tests of the longevity of spores on seed because of the uncertainty of the age of obtainable commercial seed.

Germination tests were made on spores from old specimens in the Michigan State College herbarium, but no viable spores were found. A specimen of the small type was collected in California and placed between a pair of ordinary blotters and sent to the writer through the mail. When the specimen arrived the spores did not germinate in agar and no infection resulted when the spores were sprayed on celery plants. One lot of leaves infected with the large-spot type was pressed between blotters and stored between sheets of newspaper in a drawer of a desk. After 8 months, plates were poured from these leaves and a considerable number of the spores germinated.

HOST RELATIONSHIP

Beach (3) found the members of the genus Septoria to be very limited in their host range. He made a few successful intergeneric inoculations in the same family but, in general, found members of the genus Septoria to be limited to a specific host and varieties of that host. Pethybridge (21) found a Septoria on wild celery that appeared very different from the one on cultivated celery, but upon cross inoculation the typical cultivated form developed. Thomas (28) tried the celery Septoria on several representatives of the Umbelliferae, with negative results. Laibach (18) tried his two forms of the celery organism on various members of the Umbelliferae, without success.

Since two separate species of Septoria are involved in this study, it was thought that one of them may have come from some nearly related umbelliferous host and, having found suitable environment, become pathogenic on celery. Seeds of various representatives of the Umbelliferae³ were planted in sand and the seedlings were transferred to 3-in. pots, where they grew until large enough for inoculation. Separate lots of two plants of each of the species listed in the tabulation were atomized with a spore suspension obtained from typical leaf lesions of the two types of Septoria. Four healthy celery plants were atomized and placed in infection chambers with other representatives of the Umbelliferae as checks. The plants were left in the chambers for 52 hours after inoculation and then removed to the greenhouse bench, where the temperature was nearly 20°C. The following is a list of the Umbelliferae inoculated:

Aethusa cynapium L.	Bowlesia tenera Spreng.
Ammi majus L.	Bupleurum candollii Wall.
Angelica purpurascens Lall.	" longicaule Wall.
Apium graveolens L. (Several lots)	" longifolium L.
" var. rapaceum	" rotundifolium L.
Apium repens Reicht.	" salicifolium Soland.

³ Seeds were sent to the writer from the following places: Missouri Botanic Garden, St. Louis; Royal Botanic Garden, Edinburgh; Museum d'Histoire Naturelle, Culture, Paris; Berlin Botanical Garden and Museum, Berlin; D. M. Ferry Seed Co., Detroit; Beal Botanic Garden, Michigan State College; and from local stores.

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Carum carvi L. Caucalis arvensis Huds. nodosa Gaertn. Conium maculatum L. Coriandrum sativum L. Daucus carota L. var. Oxheart grandiflora Hoffm. maximus Desf. Eryngium agavifolium Griseb. amethystinum L. 66 campestre L. " heldreichii Boiss. " planum L. wrightii Grav $Ferula\ communis\ L.$ glauca Gouan. Foeniculum vulgare Mill. Heteromorpha aborescens Cham. Levisticum officinale Koch. Oenanthe crocata L. lachenalii Gmel.

peucedanifolia Roll.

phellandrium Lam.

pimpinelloides L.

silaifolia Bieb.

Opopanax chironium Koch.

Pastinaca sativa L. var. Danvers Petroselinum sativum Hoffm. segetum Koch. Peucedanum cervaria Cusson araveolens L. officinale L. Pimpinella saxifraga L. Scandix australis L. balansae Reut. brachycarpa Guss. necten-veneris L. pinnatifida Vent. Selinum tenuifolium Wall. Seseli elatum L. gummiferum Poll. libanotis Koch. tenuifolium Ledet Silaus flavescens Bernh. Smyrnium olusatrum L. Thapsia villosa L. Thaspium aureum Nutt. Tordylium maximum L. Trachymene coerulea R. Grah. pilosa Sm. pusillus F. Muell. Trinia kitaibelii Bieb.

Successful infection was obtained with both forms only on *Apium graveolens* and *A. graveolens* var. rapaceum. The check celery plants developed typical infection in every case. Infection was attempted on several members of the family Umbelliferae by placing small pieces of agar, containing fungus mycelium, on the leaves under absorbent cotton, but no typical disease was produced.

VARIETAL RESISTANCE

The following commercial varieties of celery were tested for evidence of resistance: Golden Self Blanching (tall strain), Golden Self Blanching (dwarf), Easy Blanching, M. A. C. Yellows Resistant A. A. 25, Simon's Golden Plume, White Plume, Burpee's Fordhook, Curly Leaf, and Giant Pascal. The easy-blanching varieties were all infected and soon lost their color and died, the plants affected with the large-spot form succumbing first. The green varieties seemed to be able to endure longer the effect of both Septoria species, but 10 days after the disease became evident, the plants affected with the large-spot form began to show yellowing near the edge of the leaves.

Thomas (28) reported the younger leaves of celery to be more susceptible to Septoria than the older ones, but less easily infected, due to less surface area. In infection experiments the writer found young and old

leaves infected with equal ease. This tends to show that infection is more a matter of contact, or ability of the fungi to gain entrance, than susceptibility.

MODE OF INFECTION

Campanile (10), working with what is probably the large-spot form, described the fungus as entering the celery leaf by penetrating directly through the walls of the epidermal cells. After the fungus was established in the epidermal cells, it grew into the intercellular spaces and a characteristic spot was produced by collapse of the tissue.

Spores of both forms were placed in drops of water on the lower epidermis of celery leaves that had been removed and placed in a moist chamber. The epidermis was stripped from the leaf at different intervals and the progress of germination of the spores observed. After 3 days germ tubes were formed from various cells of the spores (Fig. 3, C to G). The first noticeable change previous to germination was the appearance of large oil droplets in the spores. The spores of the small type showed a markedly greater accumulation of oil. Spores of both types of Septoria showed an increase in the number of septa just previous to germination. In many cases the germ tubes were observed to grow directly across the surface of a stoma without entering it (Fig. 3, H, I). A single case was observed where the small slender germ tube of a spore of the small-spot type entered an epidermal cell near its anticlinal wall and branched inside the cell (Fig. 3, K).

Inoculated leaves that had been kept in a moist chamber for a period of 4 days were examined. Small hyphal systems had been established in several cells that were not bordered by stomata. Leaves inoculated on different surfaces showed the same number of spots per leaf, although the ratio of stomata per field under low power of the microscope was for upper to lower surfaces as 3.4 is to 8.2.

These observations have led the writer to believe that entrance may be through any portion of the surface of the leaf. There seems to be no stimulus that attracts the germ tubes to the stomata. Entrance through a stoma is possible, but the fungus may enter a guard cell, as well.

LEAF-STALK INFECTION

Dorogin (11) recognized typical lesions produced on the leaf stalks by the small-spot form but did not find lesions of his large-spot form on the leaf stalks. Foster and Weber (14) described their two strains of the celery Septoria as producing identical lesions on leaf stalks.

Portions of leaf stalks were washed in sterile water and placed in a sterile moist chamber. Drops of spore suspensions of each form of the Septorias were spread along different stalks. After 16 days typical lesions developed on the stalks inoculated with the small-spot form. The stalks

inoculated with the large-spot form developed very shallow brown spots, but no pycnidia were formed.

No lesions on leaf stalks were found in any of the infection experiments with the large-spot type of Septoria on celery in the greenhouse, and no such lesions were observed as the result of natural infection in the field. Lots of market celery were atomized with spore suspensions and stored at room temperature. A few lesions of the small-spot type developed, but none of the large-spot type was noted.

FUSING OF GERM TUBES

Another feature noted in culture and on leaves was the early fusing of germ tubes of germinating spores (Fig. 3, D, G, H, J). The germ tube from an occasional spore seemed to grow in a given direction until in proximity to another, when it turned shortly and fused as if attracted by some force. An occasional spore would form a single germ tube that would fuse directly with that of a similar spore, while none of the other cells in the spore germinated. If conidia are merely encysted vegetative portions of an organism, it would be only natural to expect that the germinating conidia would fuse as do other hyphae in culture. Time did not permit inoculation experiments with single and with fused conidia.

EFFECT OF LIGHT ON PYCNIDIUM FORMATION

Although pycnidia may be found on both sides of a leaf, it was observed in natural infection that they were most plentiful on the best illuminated side. To study this factor, healthy celery leaves were placed in a large glass culture dish. In a series of leaves inoculated with a drop of spore suspension on the normal top side, some were placed with the upper side and some with the under side up. A similar set-up was made with the leaves inoculated on the under side. In all cases the greatest number of pycnidia were found on the side toward the light.

GEOGRAPHIC DISTRIBUTION OF THE TWO SPECIES OF SEPTORIA

Immediately after it had been proved definitely that there were two species of Septoria attacking celery, the question of geographic distribution arose. Septoria on celery has been reported from all parts of the United States where the crop is grown, but, since only one form was recognized, the same name has been applied to both forms. Since only the small-spot form has been found on celery leaf stalks, the damage reported on celery in storage was most likely due to it. Some of the damage to celery has been accredited to Septoria petroselini Desm. It has been proved that the celery forms do not attack parsley, but lack of material prohibited a test of the parsley Septoria on celery.

From literature, specimens, and correspondence, it seems that the largespot Septoria is the common form on celery in Europe. Although the large-spot form was observed in the United States about the same time as it was in Europe, the small-spot form is the most common and destructive at the present time in the United States.

The distribution of the two forms in the United States may be indicated by the data in table 4, which is the result of a questionnaire sent to the States where celery is grown and examination of the specimens received.

TABLE 4.—Distribution of the two forms in the major celery-growing regions in the United States

	Material	Occurrence							
State	examined	Small type	Large type						
California Oregon	4 specimens 4 ''	3 specimens 2 ''	1 specimen 2 specimens						
Washington Minnesota	3 "	ı "	2 "						
Wisconsin	letter and 2 specimens	most prevalent 1 specimen	widespread 1 specimen						
Indiana	personal observa- tion	most prevalent	has been seen						
Michigan	personal observa- tion and 2	66 (1) 66 (1)	common						
Ohio	specimens 5 specimens	2 specimens 5							
New York Delaware	8 "	6 "	2 specimens						
Massachusetts	7 · · · · 3	7 "	2 "						
New Jersey Florida	2 '' and	1 "	2 "						
	Fla. Sta Bul. 173.	widespread	widespread						

NAMES OF THE CAUSAL ORGANISMS

The actual date when Septoria was first described on celery is somewhat disputed and uncertain, due to the fact that several workers described fungi resembling Septoria at about the same time. Spegazzini (24) in 1887 described a fungus on Apium australe from Tierra del Fuego and Staten Island under the name Septoria apiicola. Although this is the first record of a Septoria on Apium, it is impossible from the description to tell whether the fungus is identical with either of the two on cultivated celery. It is possible that, upon examination of specimens of the fungus on A. australe and after cultural and inoculation studies, this may prove to be the same as one of the celery Septoria species, but, until such studies can be made, it seems advisable to maintain it as a separate species. Briosi and Cavara, in 1890, described a fungus on čelery in Italy, which they

called Septoria petroselini Desm. var. apii. They collected and distributed it with descriptions as No. 144 in I Funghi Parassiti. They retained the specific name because of its likeness to the Septoria on parsley; yet, by the varietal name, indicated the difference between the celery and the parsley organisms.

At about the same time Allescher collected specimens of a fungus on celery in Bavaria, which he sent to Bresadola. Allescher, in his letter, called the fungus Septoria Magnusiana, but in describing the fungus, Bresadola noted some subhysteroid ostioles and apparently imperfect pycnidia and transferred the fungus from Septoria to Phlyctaena. These specimens collected by Allescher were distributed under Bresadola's name Phlyctaena magnusiana (Allesch.). Bres. as No. 188 of Allescher et Schnable, Fungi Bavarici. This set was dated 1890 but was not distributed until 1891. Accompanying the specimen No. 188, there was a printed description of the new species, so that the publication of the specific name magnusiana dates from early in 1891, when the set was distributed. The species description was published by Allescher (1) in 1892.

Chester (4, 5), in Delaware in 1891, noted the presence of the fungus on celery and identified it with Septoria petroselini Desm. var apii. Br. and Cav. He compared the form on celery with the true S. petroselini Desm. on parsley and decided that the differences were sufficient to warrant raising the variety apii to specific rank. He published the name S. apii (Br. et Cav.) Chester (5) in the bulletin of the Torrey Botanical Club, Dec. 9, 1891. According to Klebahn (16), Rostrup in 1893, ignorant of Chester's work, also described the fungus under the name Septoria apii Rostrup.

The name magnusiana is the first specific name applied to the fungus and according to the rules of nomenclature, would be the correct name to use, except for the fact that Allescher subsequently, in 1892, used the combination Septoria magnusian Allescher for an entirely different species on Spiraea chamaedryfolia. Thus, the earliest available name for the celery organism is Septoria apii (Br. and Cav.) Chester.

According to Klebahn (16) and Dorogin (11), who examined the specimens in the sets of exsiccata, Septoria petroselini Desm. var. apii Br. and Cav. is identical with Phlyctaena magnusiana (Allesch.) Bresadola. The present writer has compared Chester's original specimens (sent through the kindness of Dr. Manns) with those of Briosi and and Cavara and confirms Chester's findings.

From the description and specimens of Briosi and Cavara, Klebahn, and Chester it is clear that the fungus studied by them is identical with the large-spot type studied by the writer. Although the specimens of Briosi and Cavara are identical with the large-spot type, the descripion accompanying the specimens does not exactly fit the specimens. The pycnidia

are described as crowded and fusing. It seems quite possible that both types of Septoria may have been present in Italy and the small-spot type may have confused the description, the author not knowing that there were two separate Septorias attacking celery. The name Septoria apii (Br. and Cav.) Chester then applies to the causal organism of the large-spot type of the disease.

In 1915 Dorogin (11) described two distinct Septorias as parasites on celery. One type he described as causing a large spot, reddish brown, and, when mature, having minute pycnidia sparsely scattered near the center of the spot. The pycnidia were nearly round, paraplectenchymatous in nature, $70-125\mu$ in diameter; the ostiole $\frac{1}{5}$ to $\frac{1}{4}$ the diameter of the pyenidium and round or irregular in form. The spores were filiform, 30 to 40 $\mu \times 1.5$ to 2 μ , about straight without visible granules, and with 3 inconspicuous septa. No pycnidia were found on the stalks. This form he identified with the Briosi and Cavara and Chester type, i.e., Septoria apii (Br. and Cav.) Chester. The other he described as causing a small spot with densely crowded, often fused, black pycnidia. The pycnidia were 90 to 135 u in diameter, paraplectenchymatous in nature and characterized by very large ostioles often somewhat slit and sunken in the uncollapsed tissue around the spot. The spores were $45-50\times2-3.5\,\mu$, obtuse at the ends, distinctly granular and as many as 6-septate. Lesions with crowded pycnidia were abundant on the leaf stalks. He concluded that this second fungus was a new species and gave it the name Septoria apii-graveolentis.

Laibach (18) in 1921, in reviewing the work of Dorogin and reporting his own work, considered the two types of the celery late-blight organisms as varieties of the species Septoria apii. He gave the names S. apii var. maculiformis and S. apii var. punctiformis to the large- and small-spot types, respectively. His reason for this was that he observed saltations in his cultures in which both forms were represented. From pictures of the disease on leaves and pictures of the cultures in Petri dishes, it appears that the two fungi he described are similar to those of this study. However, since pure cultures of the organisms showed no such saltation and always produced typical forms of the disease for the writer, and the spores are quite different in size and shape, and the physiological reactions of the organisms are markedly different, the writer agrees with Dorogin in regarding the two forms as separate species.

One specimen mentioned by Dorogin as examined and found identical with his Septoria apii-graveolentis was collected in London, Ontario, Canada, by Dearness. A specimen of this same collection (Fungi Columbiani) in the herbarium of Michigan State College was found to be identical with the small-spot type occurring in Michigan. The name of the causal organism of the small-spot type then should be Septoria apii-graveolentis Dorogin.

SUMMARY

- 1. Two distinct species of Septoria were isolated from late-blight lesions on celery and definitely proved to cause different types of disease.
- 2. The two diseases on celery are characterized by large and small lesions and may be distinguished accordingly. The large-spot lesion is regular in outline and has small, black, nearly spherical pycnidia scattered singly in its central portion. The small-spot lesion is indefinite in outline and has pycnidia of nearly twice the size of those of the large-spot type. The pycnidia of the small type may be found in both collapsed and uncollapsed tissue, occurring singly or fused in twos and threes. Fruiting bodies are never found in the large-type spot until a definite region of tissue has collapsed and even then only under conditions of moderate to high humidity.
- 3. In pure culture the two species of Septoria grew well on most laboratory media, but the type of growth was characteristically different. The large-spot organism made a spreading growth with black submerged mycelium and white aerial mycelium. The small-spot organism made a more tufted growth of somewhat cheesy consistency. On potato-dextrose agar, the large-spot organism produced a purple color in the medium around the colony.
- 4. Spores of the small- and large-spot organisms were killed in 10 minutes at 41° C. and 43° C., respectively. The optimum temperature for growth of the small-spot fungus was between 18° and 22° C., while that for the large-spot one was between 22° and 24° C.
- 5. A large number of the family Umbelliferae were inoculated with both fungi, but infection resulted only on *Apium graveolens* (celery) and its variety rapaceum (celeriac).
- 6. Several varieties of commercial celery were inoculated and, although the green celeries seemed more able to endure the diseases, all seemed to be equally susceptible.
- 7. Although both species of Septoria are widely distributed in the United States, the name generally used in literature and applied to specimens in herbaria is that of the large-spot organism. Geographic studies showed that the fungus causing the large spot is probably the common one in Europe, but the small-spot-producing organism is far more common in this country.
- 8. The small-spot type of disease is caused by Septoria apii-graveolentis Dorogin. The large-spot organism is Septoria apii (Br. and Cav.) Chester.

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SOME ENVIRONMENTAL RELATIONS OF WATERMELON WILT

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INTRODUCTION

The intimate relation of such variables as temperature, moisture, and soil reaction, sometimes termed "conditioning factors," to the occurrence and severity of certain plant diseases is well recognized. Fluctuations among these factors doubtless exert either direct or indirect influences on both the host and parasite. While the effect of each variable probably never can be completely segregated and while the environmental conditions under which plants are grown experimentally cannot be exactly duplicated under field conditions, yet the results as reported by various workers have been remarkably consistent with respect to the behavior of the diseased plant when subjected to different environmental conditions.

It is the purpose of this paper to set forth indicative evidence relative to the effects of such conditioning factors as air temperature, air humidity, wind velocity, and sunlight intensity on the rate of death of watermelon plants growing in wilt-infested soil. Taken collectively, these effects were measured by the rate of water evaporation from atmometer cells. Taken singly, some effects of all except wind velocity are indicated. These studies were made in Iowa in 1927 and 1928 and at Davis, California, in 1931.

HOST RESPONSE

Watermelon wilt, the vascular disease caused by Fusarium niveum E. F. Sm., is manifested by slightly different host response from that of vascular fusarioses of tomato, cabbage, cowpea, and cotton. With the latter plants, death does not always quickly follow the first manifestation of infection, but diseased plants may cease to manifest symptoms, partially recover, or even reach maturity before the plants die. A wilt-infected watermelon plant usually dies within 10 days after the first sign of disease becomes evident. In many cases infected plants die within 24 to 48 hours after wilting begins. Seedling plants may succumb with 2 to 6 hours after the cotyledons begin to droop. While the reasons for this diversity of host response are not known, it is suspected that the greater leaf area of the watermelon plant, with consequent rapid and extensive water loss might offer a partial explanation. Furthermore, the more woody nature

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of such plants as tomato, cotton, sweet potato, and cabbage may influence their relatively slow rate of death following infection.

Observations indicated that with an abrupt rise of air temperature in the field, the rate of death in watermelons increased. Counts made at 6:00 A. M. following a cool night, compared with similar counts following a warm night, showed that in the former case infected plants had often temporarily recovered. In the latter case, infected plants were dead. Was recovery due to decreased transpiration from the infected plant or was it the result of low temperature effects on the pathogen? When watermelons were planted in infested soil during cool weather, seedling wilt was of rare occurrence. If the weather remained cool for several days following emergence, wilting was correspondingly retarded. When, however, planting was delayed until the soil became warm and when a period of warm weather followed emergence, seedling wilt was rapid. Was this entirely due to temperature effects on the host and pathogen or could it be correlated with the rate of evaporation from the leaves? The temperature relations are known (8) and investigation has shown (9) the relation of soil temperature to rate of wilting and manifestation of symptoms under controlled greenhouse conditions, but critical examination of interrelated environmental effects in the field has not been made.

RELATED WORK

The literature on the relation of environmental factors to the occurrence and severity of fusarial wilts has been reviewed so often of recent years that further discussion is unnecessary. Attention is directed to a few papers in which more detailed discussion may be found. There is an extensive paper by Jones et al. (6), dealing with the relations of soil temperature to several plant diseases. There are also papers by other workers as follows: Gilman (3), Tims (11), and Walker and Smith (13) on cabbage yellows; Clayton (1, 2) on tomato wilt; Harter and Whitney (4) on sweet-potato stem rot; Linford (7) on pea wilt; Tisdale (12) and Jones and Tisdale (5) on flax wilt; Young (14) on cotton wilt; and by Porter and Melhus (10) on watermelon wilt. In general, it may be stated that the vascular fusarial diseases are probably limited in their geographical distribution and seasonal severity by low soil temperature.

METHODS AND MATERIALS

Soil. The soil used in the trials in Iowa and California was known to be heavily infested with Fusarium niveum, as previous watermelon crops of commercial varieties had been almost a complete failure because of wilt. The soil used in California was of a heavier type and contained much less sand than the soil in Iowa. Artificial irrigation was practiced only in California.

Seed. For the trials in Iowa, seed of 4 commercial varieties was used, namely, Kleckley Sweet, Halbert Honey, Tom Watson, and Thurmond Grey. In the California trials, seed of the commercial variety Klondike was used. All seed was disinfected before planting.

Culture. In Iowa, the seed was planted in rows 6 ft. apart with approximately 4 ft. between the hills in the row and at the rate of approximately 15 seeds per hill. Seed was planted on top of a ridge under which commercial fertilizer and manure has been applied. In California, seed was sown on raised beds with a furrow between to facilitate irrigation. The seeds were planted approximately 2 in. apart, planting being purposely delayed until May 15 so that the soil temperature would be at or near optimum for growth of the causal organism and infection of the plants. Previous experience indicated that many of the plants would die before producing the 4th leaf, hence close planting would not result in crowding of the plants. The land was irrigated 2 days before planting and daily during the course of the experiment.

Standard spherical atmometers were used for measuring the rate of evaporation. Four cells, 2 white and 2 blackened with a mixture of lamp black, collodion, and ether were used at Davis. The bottles that supported the atmometers were placed in a hole in the ground so that the cells would be near the soil surface at approximately the same elevation as the water-melon foliage. Distilled water was used and the readings of water lost were made at 7:00 A. M. every day from May 24 to June 25, inclusive. Each morning the white cells were washed and the black cells gently rubbed with cotton moistened with distilled water. Twice during the period indicated the black cells were recoated with the lamp-black mixture. Similar apparatus was used in the Iowa experiments, but a mixture of lamp black and water, instead of the collodion mixture, was used to blacken the cells.

Six separate trials were conducted in Iowa from June 23 to September 3. The trials differed from one another in location of the fields, age of the plants, time of the year, and length of the period considered. Trial No. 1 included 4 periods of 96 hours' duration, each; No. 4 included 3 periods; while trials Nos. 2, 3, 5, and 6 were divided into 2 periods, each. In general, seedlings were not considered, the counts being based on the rate of wilting in older plants. Stand counts were made at regular intervals and as the atmometers were read daily it was possible to determine the average daily rate of evaporation for any period desired. Whenever stand counts were made, wilted plants were pulled and discarded.

One test, divided into 16 periods, was conducted in California. At the time of daily readings of the atmometers, the total number of plants was counted and the wilted plants were pulled and discarded. The percentage of wilted plants was determined from the daily counts.

RESULTS

Iowa Tests. The results of the tests in Iowa appear in table 1. With each of the 15 periods considered, the white atmometer evaporation, B-W,² and percentage of plants wilted are indicated. In addition, the length of each period, with inclusive dates, is shown and the maximum and minimum air temperature indicated. Counts made before June 23 were not considered, and the soil temperature after that date was at or near the optimum for fungus growth and infection.

Test No. 1 (periods 1 to 4, inclusive). This test continued from June 23 to July 8, inclusive, each period being of 96 hours' duration. The average percentage of wilt and evaporation rate, per 24 hours, was determined by dividing the respective totals for each period by 4. By standardizing the period duration at 24 hours in the Iowa tests, the data are summarized in one table rather than in six. Neither the rate of wilting nor the rate of evaporation varied greatly during test 1, but during period 2 both evaporation and wilting rates were higher than during the other 3 periods and the maximum and minimum air temperatures were higher than during periods 1 and 3.

Test No. 2 (periods 5 and 6). This test was divided into 2 periods of 168 hours, each, and extended from June 27 to July 10, inclusive. Both evaporation and rate of wilting were lower during the latter period, with no appreciable difference in maximum and minimum air temperatures per period.

Test No. 3 (periods 7 and 8). This test continued from July 17 to 28, inclusive. The evaporation rate was higher but the wilting rate was lower during period 7 than during 8. This is the only case in the 6 tests of apparent negative correlation. As the 2 periods were of unequal duration, such might be the explanation. It should be noted, however, that the value of B-W and the maximum and minimum temperatures were higher during the latter period.

Test No. 4. This test was divided into periods 9, 10, and 11 and extended from July 17 to 27, inclusive. By comparing evaporation with wilting rates during successive periods, it is evident that any marked change in evaporation rate was accompanied by a similar change in rate of wilting. Both evaporation and wilting rates were much lower during period 10 than during 9, but during period 11 the evaporation rate increased over period 10 with a corresponding increase in rate of wilting. Similar comparisons are evident between wilting rate and B-W.

² The difference in rate of evaporation between the black and white atmometer is indicated as "B-W." This value is a measure of the relative light intensity.

TABLE 1.-The relation of rate of evaporation and sunlight intensity to the rate of watermelon willing due to infection with Fusarium niveum. Date from tests conducted in Iowa in 1927

Air temperature in degrees C. ximum Minimum	Minimum	14 13 13 18	13 13	13	13 13 17	9 16	13
Air tem in deg	Maximum	30 35 35 35	3 3 3 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5	80 80 50 50	89 89 60 10	31 34	33 44
Average per- centage wilted plants per 24 hours of each period		1.3 1.1 1.8	2.4	9.8 8.8 8.8	4.5 1.4 9.6	6.5 15.2	4.9 9.4
aporation 24 hours	B-Wa	16.1 21.7 17.8 20.0	19.3 18.2	12.7 20.1	24.8 11.5 20.1	11.2	15.3 20.6
Average evaporation in cc. per 24 hours of each period	From white atmometers	39.8 60.5 34.3 39.7	51.6 37.3	26.3 18.7	41.3 10.3 18.5	27.5 32.2	28.4 35.3
Period	Inclusive dates	June 23–26 '(27–30 July 1–4 '(5–8	June 27-July 3 July 4-10	July 17–24 (, 25–28	July 17–19 (20–23 (24–27	Aug. 28-30	Aug. 30–31 Sept. 1–2
	Hours' dura- tion	96 96 96	168 168	192 96	72 96 96	22	48
	No.	H 62 65 4	10 9 	- 8 - 8	10 10 11 11 11 11 11 11 11 11 11 11 11 1	113 13	{ 14 { 15
Tost	No.	1	¢1	m	च	ເລ	9

This value is ^a The difference in rate of evaporation between the black and the white atmometers is indicated as B-W. measure of relative light intensity. Test No. 5 (periods 12 and 13). This test began on August 28 and terminated on September 2. Rate of wilting as well as the evaporation rate was more rapid during the latter period. Similar data are presented for test No. 6 (periods 14 and 15), which extended from August 30 to September 2. While there was a constant relation between evaporation and wilting rates during tests 5 and 6, there was also a constant relation between wilting rate and B-W.

It should again be pointed out that the 6 tests are not comparable with each other, as they were conducted in different fields, at different dates, and with plants of varying age. Within each test only successive periods are comparable. The important consideration involves the effect of a marked change in evaporation upon the rate of wilting. The relation of evaporation to rate of wilting is indicated graphically in figure 1, A, while the relation of B-W to rate of wilting appears in figure 1, B.

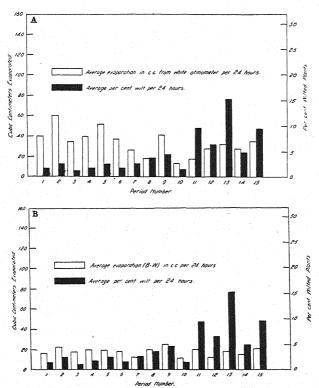


Fig. 1. A. Relation of rate of water evaporation from white atmometer cells to the rate of watermelon wilting due to infection with *Fusarium niveum*. It is possible to compare only 2 successive periods for reasons stated in the text. B. Relation of sunlight intensity to rate of wilting. Sunlight intensity was determined from the difference in evaporation rates between black and white atmometers. All data taken from table 1 (Iowa trials, 1927).

While the periods are too few in number to attempt statistical correlation, the constant tendency for relatively rapid wilting to accompany periods of relatively high evaporation rate indicates that positive correlation exists. The application of statistical formulae to environmental effects that are influenced by many separate conditioning factors might not be so accurate as data that show merely a consistent tendency in one direction.

California Tests. Data derived from the tests in California are tabulated in 16 periods of 48 hours, each, beginning May 23 and terminating on June 23. Unlike the Iowa tests, seedlings were mainly considered, although, by June 23, many of the plants had just started to form runners. In addition to the average evaporation from both black and white cells, the following records were also secured: the average soil temperature at depths of $\frac{1}{2}$, 3, 6, and 12 in.; the relative humidity of the air at 12:00 a. m.; and the mean, maximum, and minimum air temperatures. These data are presented in table 2.

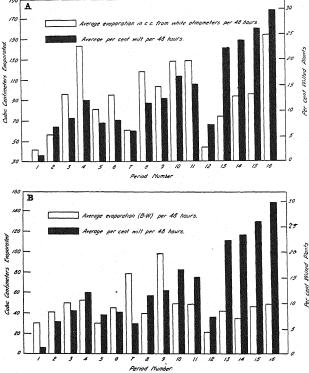


Fig. 2. A. Relation of rate of evaporation from atmometer cells to rate of wilting. B. Relation of sunlight intensity to wilting. All data taken from table 2 (California trials, 1931).

TABLE 2.—The relation of rate of evaporation and sunlight intensity to the rate of watermelon willing due to infection with Fusarium niveum. Data from tests conducted at Davis, California, in 1931

Relative humidity at 12:00	A. M. average for 2 days	52.5	62.0	51.5	30.5	42.0	50.0	37.5	46.0	47.5	39.0	36.0	60.5	47.0	40.0	42.0	30.0
cure C.	Mini- mum	13	10	П	П	10	6	11	Π	10	10	П	Π	∞	П	П	6
Air temperature in degrees C.	Maxi- mum	28	56	37	38	31	82	34	31	87	32	34	24	53	28	33	33
Air	Mean	15	16	23	23	15	16	23	20	17	19	. 50.	18	91	18	19	23
ture	12″				56	56	25	25	25	25	56	27	25	24	24	25	27
Average soil temperature in degrees C.	<i>"</i> 9		1	-	27	56	25	56	97	56	27	28	24	24	25	27	28
age soil in deg	%G				58	28	25	56	56	22	82	59	23	24	56	27	28
Aver) 			1	28	56	56	87	22	22	53	53	24	24	25	58	28
Percent-	wilted plants	1.2	6.4	8.6	12.1	2.8	8.2	6.1	11.5	12.4	16.6	15.1	7.2	22.2	23.3	26.1	29.7
ration in cell	B-Wa	31	42	51	53	31	47	7.9	41	86	48	49	22	42	35	47	48
Average evaporation ec. from 1 cell	From white cell	42	57	26	144	85	96	62	118	104	128	129	44	73	93	96	154
Average ec.	From black cell	73	66	148	197	113	143	141	159	202	176	178	99	115	128	143	202
Period	Inclusive	May 23-24	25-26	27-28	29-30	31-June 1	2-3	4-5	2-9	6-8	10-11	12-13	14-15	16-17	18-19	20-21	22-23
	No.	T	c 1	က	4	ro ro	9	2	∞	6	10	11	12	13	14	15	16

a Indicates relative sunlight intensity.

During 15 of the periods, when the rate of evaporation increased there was an increase in rate of wilting. During period 1 the average evaporation was 42 cc. with only 1.2 per cent wilt, but during period 2 the average evaporation was 57 cc. with 6.4 per cent wilt. Similar relations are apparent when comparing periods 4 with 5, 7 with 8, 11 with 12, 12 with 13, and 15 with 16. Correlation of white-cell evaporation rate with rate of wilting is clearly evident from the graphical representation in figure 2, A, when successive periods are compared.

The relation between rate of wilting and sunlight intensity, represented in table 2 and figure 2, B, as B-W, is also well expressed. During 10 of the 16 periods positive correlation apparently existed. In most cases periods of increased sunlight intensity were accompanied by more rapid wilting than during the preceding or succeeding period of less intense sunlight. During period 11 the value of B-W was 49 cc. and the percentage of wilt was 15.1, but during period 12 the value of B-W decreased to 22 cc. and the percentage of wilt to 7.2. Similar relations are apparent when comparing periods 2 with 3, 4 with 5, 12 with 13, and 14 with 15.

AIR TEMPERATURE

A brief statement of the relation of air temperature to rate of wilting ing the field was reported previously by the writer (9). The following data and discussion constitute a more detailed report.

The relation of air temperature to the rate of wilting was studied in 2 infested fields at Conesville, Iowa, in 1927 and 1928 and in a field at Davis, California, in 1931. The soil used was so heavily infested with the wilt fungus that commercial crops suffered more than 99 per cent mortality. In Iowa, disinfected seed of the varieties Kleckley Sweet and Tom Watson was planted at intervals from May 20 to July 1, stand counts being made from the date of emergence until the end of the growing season. The difference in the number of living plants at the time of succeeding stand counts was assumed to be due to wilt. The percentage of wilt thus computed was then divided by the number of days that had elapsed since the last reading giving "average per cent daily wilt since last reading."

The air temperature was recorded automatically by a Friez thermograph. The total number of hours that the air temperature was above a certain point was determined for the interval between the dates of successive stand counts. This number was then divided by the number of days that had elapsed since the previous reading, giving the average number of hours per day that the air temperature was above 24°, 27°, 29°, 32°, and 35° C.

The relation between air temperature and rate of wilting is presented graphically in figure 3, considering only the relation existing at tempera-

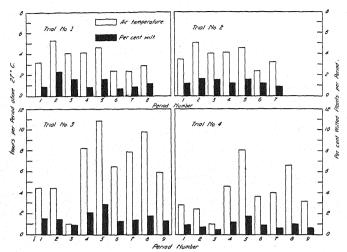


Fig. 3. The relation of air temperature above 27° C. to rate of watermelon wilting due to infection with Fusarium niveum. (Similar data are available with respect to temperatures of 24°, 29°, and 32° C.) Only successive periods of each trial may be compared. The tendency is for rapid rate of wilting to parallel sustained periods of warm weather under field conditions.

tures at or above 27° C. Similar charts were prepared for 24°, 29°, and 32° C., and, as the same general tendency was evident, only one chart is included herewith. It should be stated that the nature of the data is such that different tests should not be compared, the reason being that each test extended over a month or more. Comparisons should be made only between 2 successive periods of the same test. Considering test 1; the percentage of wilted plants during periods 1 and 2, respectively, was 0.9 and 2.4; at the same time the air temperature was above 27° C. for 3.3 hours during period 1 and for 5.5 hours during period 2. During period 3, the percentage of wilted plants decreased to 1.8 and the total hours above 27° C. to 4.2.

In test 2 the relation seems somewhat less evident than in test 1, but there is the same general tendency. A striking correlation is apparent when subsequent periods of test 3 are considered. The abrupt and sustained rise in air temperature during period 4 and accompanied by an increase in the rate of wilting from 0.9 per cent during period 3 to 2.1 per cent during period 4. Even warmer weather prevailed during period 5 than during period 4, with an accompanying increase in the rate of wilting. During period 6, cooler weather than during period 5 prevailed and rate of wilting decreased. Parallel instances are also evident during trial 4, particularly when period 3 is compared with 4, 5 with 6, 7 with 8, and 8 with 9.

In general, the data indicate that with relative increase in the number of hours of warm weather the rate of wilting increases. It is not known whether this increase in wilting rate is due to abrupt rise in air temperature or to sustained high temperature. An abrupt rise in temperature would lower the relative humidity, since warmer air can hold more water. This would favor wilting. When the 32-degree chart was examined it was apparent that the relation was very similar to that expressed for 27° C. in figure 3. Obviously, during the same period, the air temperature would be above 32° C. for a much shorter time than above 27° C., yet the relations to wilting rate were approximately the same. It is possible that either an abrupt weather change or a sustained period of changed weather conditions would induce like effects. In addition, the relations seem to hold whether seedlings or more mature plants were considered.

In California, air temperature, soil temperature, and relative humidity were determined during the period that the evaporation rate was being measured. The relation of these factors to the rate of wilting is indicated in table 2, attention being directed particularly to the relations between the mean air temperature and the rate of wilting when successive periods are compared. It appears that during 10 of the 16 periods correlation is positive. These findings agree with those of the Iowa tests. As the correlation between rate of wilting and rate of evaporation from white cells (Fig. 2, A) is much closer than that from mean air temperature (Table 2), it is evident that such additional conditioning factors as wind velocity, air humidity, and sunlight intensity exert some influence. The data on the effects of sunlight, indicated as B-W, are included in table 2 and in figure 2, B. The humidity relations indicated in table 2 are less conclusive, but association of low humidity with high wilting rate was evident in 8 of the 16 periods.

It should be noted that the average soil temperature per period at depths of $\frac{1}{2}$, 3, 6, and 12 in. was never below 74.5° F., and, as infection may occur at or above 68° F., it may be concluded that the average soil temperature during the course of the experiment was such that infection could take place.

DISCUSSION

The investigations herewith reported were not conducted under controlled conditions; they were conducted in the field in an attempt to measure the cumulative effects of variation in natural environmental conditions on the rate of death of watermelon plants growing in soil heavily infested with *Fusarium niveum*. While it is recognized that more positive conclusions result when environmental conditions are controlled, yet the results presented herewith indicate what happened under field conditions.

In general, those external conditions of environment that determine the rate of transpiration from leaves and the rate of evaporation from atmometer cells appear to influence the rate of wilting of watermelon plants infected with Fusarium niveum. In these experiments, the soil-moisture supply was adequate for noninfected and for resistant plants; thus deficient moisture supply alone was not responsible for wilting. Whether infected plants were able to absorb sufficient water from the soil is not known; furthermore, the transpiration rate from them was not determined. Working with pea wilt (F. orthoceras var. pisi), Linford (7) presented some evidence that wilting resulted from excessive water loss from the leaves of infected plants, and not entirely from a diminished water supply.

The data herewith presented indicate that variation in the rate of wilting depends to some extent upon the variations in those conditioning factors that influence transpiration. Combinations of increased light intensity, abrupt or sustained rise in air temperature, and low relative humidity increase the rate of transpiration. The data indicate that these conditioning factors exert similar effects on rate of wilting. It seems evident, therefore, that rate of wilting is determined by the extent of interference with water movement in the plant. These experiments support the theory that the presence of the fungus in the conducting tissues interferes with water movement. It is not known whether this interference is due to mechanical obstruction by the mycelium and spores, to indirect influences of the pathogen, or to a chemical substance formed by the fungus.

SUMMARY

The rate of watermelon wilting due to infection with *Fusarium niveum* is influenced by such environmental factors as air temperature, relative humidity, sunlight intensity, and wind velocity.

Tests conducted in Iowa in 1927 and 1928 showed that there is a tendency toward positive correlation of the air temperature with the rate of wilting. Similar conclusions are drawn from the results of trials conducted in California in 1931.

Sunlight intensity also seems to have some relation to the rate of wilting, the tendency being for relatively rapid wilting to follow periods of relatively intense sunlight as determined by the difference in the rate of evaporation between the black and white atmometer cells. Although this rate was much higher in California than in Iowa, the same general conclusions may be drawn. From the California tests it is concluded that the rate of wilting increased with a decrease in the relative humidity of the air.

The rate of evaporation as indicated by white atmometer cells, influenced by air temperature, air humidity, light intensity, and wind velocity, ap-

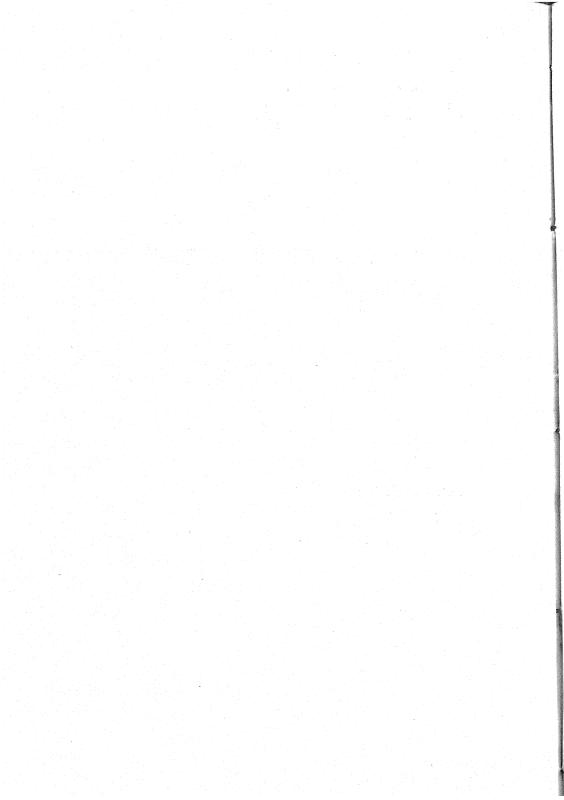
pears to influence the rate of wilting. With increase of evaporation rate, the rate of wilting increases. Relatively slow rate of wilting occurs during periods of relatively slow rate of evaporation.

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RHIZOCTONIA SOLANI ON CEREALS IN SOUTH AUSTRALIA

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Although Rhizoctonia solani Kühn is known to have a very wide host range and has been reported by Rayllo (5) as affecting wheat and oats in cross-inoculation studies with the potato strain of the organism, yet so far this fungus does not seem to have been reported as causing a definite disease of cereals in the field. It is true that Rhizoctonia has been identified, though usually as one of a number of fungi, in isolations from foot rots of wheat in several countries. In these cases, however, the disease has not always been definitely attributable to the Rhizoctonia, and the identification of the fungus has usually not been carried so far as the species. One of the most definite cases on record appears to be the occurrence of a Rhizoctonia on wheat in India, ascribed by Subramaniam (9) to Rhizoctonia destruens Tassi, which was considered to be the same fungus as Sclerotium rolfsii Sacc. Godfrey (3) also reported the latter fungus on wheat in the United States. Shaw and Ajrekar (8) have also reported Rhizoctonia napi West on wheat and oats in India. Peyronel (4) isolated a species of Rhizoctonia, which he considered was possibly a simple variation of R. solani, from the base of the culm and from roots of wheat affected by root rot in Italy. Fairly definite statements have also been made of Rhizoctonia causing root rot of wheat by Bessey¹ and by Richards² in the United States, but in neither case was a description, either of the disease or of the fungus, given.

In a preliminary note Samuel (7) reported a Rhizoctonia as causing a definite seedling disease in wheat and oat fields in South Australia, and the object of the present paper is to give a fuller description of this disease.

THE DISEASE

The disease affects wheat, oats, barley, and pasture plants on what is known as the "mallee" soils of southern Australia (Fig. 1). These soils take their name from the native vegetation, which was a dwarf Eucalyptus scrub known as mallee. They are usually characterized by alternating sand ridges and red-loam flats overlying a shallow limestone. They are decidedly alkaline, mostly having a reaction between pH 8.0 and pH 9.0.

¹ Bessey, E. A. Rhizoctonia root rot of wheat. U. S. Dept. Agr., Bur. Plant Indus., Plant Dis. Bul. Sup. 8: 37. 1920.

² Richards, B. L. Root rot of wheat. U. S. Dept. Agr., Bur. Plant Indus., Plant Dis. Bul. Sup. 27: 207. 1923.

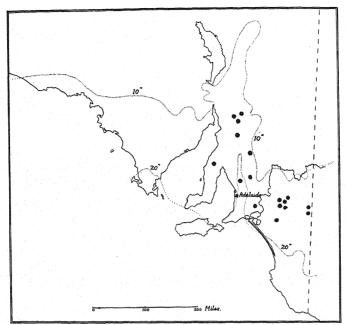


Fig. 1. Map of the southern portion of South Australia, showing localities from which the seedling disease of wheat and oats, caused by *Rhizoctonia solani*, has been reported. The area enclosed by the 10-in. and 20-in. rainfall isohyets roughly delimits the mallee soils.

The average annual rainfall is from 12 to 18 in., falling mainly in the winter.

The disease itself is not of great economic importance. It occurs in patches that vary from 2 ft. or less in diameter to large irregular areas an acre or more in extent. Small patches some 3 to 6 ft. across are perhaps the commonest. Usually only an odd patch or two can be found in a crop, but occasionally the infected spots are more numerous and coalesce into larger areas. The margins of the spots are usually sharply defined; they become very noticeable as the crop grows up, owing to the hollow space left, with small stunted seedlings that usually die later, the space becoming filled with weeds by harvest time. In a number of cases the patches have persisted in the same spots in the field over several years and have even enlarged somewhat, in spite of a year's fallow.

Symptoms. The diseased plants are noticeable at a very early stage, frequently before the crop has begun to tiller. They appear spindly and stunted, with stiff, rolled leaves pointing upwards (Fig. 2, A). In the case of oats there is often considerable purpling of leaves and stems, especially later, when dry weather sets in. The affected plants do not tiller and

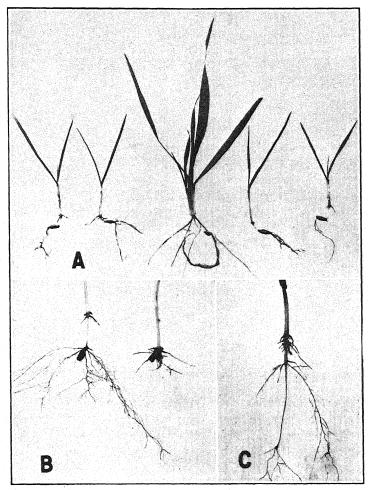


Fig. 2. A. Oat seedlings, showing the characteristic stiff and stunted appearance due to the attack of the roots by *Rhizoctonia solani*. Healthy plant in the center. B and C. Wheat and oat roots, respectively, showing killing back of both seminal and nodal roots by *R. solani*.

usually remain practically at a standstill during the winter months of June, July, and August. Then, as the weather becomes warmer, all the plants in many of the patches die without having attained a height of more than about 6 in. In a few cases, when the attack is apparently not so severe, some of the plants are able to form fresh roots with the advent of the warmer weather and may make a fairly good recovery. This may happen with isolated plants in the patches or occasionally with all the plants in a patch. In the latter case the patch is noticeable when the crop is ripening

in November as an area where the plants are still green, owing to the delay in maturity caused by the initial check given by the fungus. By far the most common effect of the disease, however, is the killing of all the plants when still very small, so that by harvest time the "hole" in the crop is filled with weeds.

It is the roots of the plants that show the most characteristic symptoms. If diseased plants are dug up in the earliest stages and their roots washed out, it is found that the tips or intermediate portions of the root are so vigorously attacked that the tissue loses all turgidity. The effect is very reminiscent of "damping-off" on the stems of crowded herbaceous seedlings, but in the case of the cereals it is only the roots that have been found affected, and never the stem, even below ground.

The flaccid, water-soaked appearance of the tissues in this earliest stage does not last long. The cortex rots away and the central cylinder breaks, so that the affected part is soon nothing but a brown stub (Fig. 2). The killing of root tips and other parts of the roots stimulates the formation of laterals, which are in turn attacked. The result is a rather characteristic, much-branched root system in diseased plants, mainly in the seminal roots, for the roots put out from the first node are usually quickly killed back. This, in fact, is largely the reason for the severe effect of the fungus on the plants, for they are unable to establish any secondary root system.

THE CAUSAL ORGANISM

When the flaccid portions of the root seen in the earliest stages of the disease are examined under the microscope the cortical tissues are seen to be penetrated by abundant stout, vigorous, colorless hyphae full of a clear refringent protoplasm. This stage is very transitory, however, and by the time the root has rotted back to a stump it is almost impossible to find any active-looking hyphae at all. Corresponding with this, it is easy to isolate the fungus during the first stage but very difficult to obtain it at the later stage, when saprophytic fungi and bacteria are already established on the browned stub of the root. At this later stage occasional long, thin, dark brown hyphae with typical Rhizoctonia branching are often seen in the soil outside the root.

The vigorous intracellular hyphae average $10\,\mu$ in diameter, but when passing directly across cortical cells they may attain a diameter of $17\,\mu$, mainly because they are somewhat swollen just preceding the constriction for penetrating the cell walls (Fig. 3). At the height of their activity these hyphae may be so packed inside the cortical cells that they seem almost to have replaced the entire cortex. With the disintegration of the tissue the hyphae rapidly become vacuolate, and when the root has been reduced to a stub the long, thin, brown, distributive hyphae $7\,\mu$ in diameter

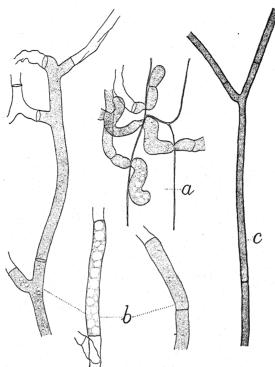


Fig. 3. A. Mycelium of *Rhizoctonia solani* in cortical cells of wheat root. Hyphae colorless and full of clear, refringent protoplasm. B. Stages in rapid vacuolation and emptying of these hyphae. C. Thin, brown ''distributive'' hyphae found in the soil surrounding roots after disintegration of the cortical tissues.

are the only ones to be seen. These are the hyphae that evidently grow through the soil and cause fresh infections when they come in contact with healthy roots. The origin of the brown distributive hyphae from the colorless, actively parasitic hyphae and, *vice versa*, have both been seen.

Isolations have been made from diseased patches in widely scattered localities, and in all cases a fungus has been obtained that on potato-dextrose agar gives a vigorous light brown mycelium of characteristic Rhizoctonia character, which later forms rather dark brown sclerotia of irregular size and shape. This fungus has been compared with 3 strains of *Rhizoctonia solani* isolated from potatoes and, as shown in the following paragraphs, is practically indistinguishable from these strains. Of the 3 strains of *Rhizoctonia solani* used, 2 were kindly supplied by Dr. W. L. Waterhouse, 1 having been isolated from New South Wales potatoes and the other received by him from Minnesota, U. S. A. The 3rd strain was freshly isolated from sclerotia on South Australian potatoes.

COMPARISON WITH RHIZOCTONIA SOLANI FROM POTATOES

(1) Growth on potato-dextrose agar at different temperatures. The 2 isolates compared in this test were No. 161, Rhizoctonia from wheat, Tarcowie, South Australia, and No. 171, Rhizoctonia solani from potatoes, New South Wales. The experiment was carried out in a multiple temperature incubator with 14 compartments. Each strain was grown in triplicate at each temperature; the inoculum was from mycelium from colonies previously grown at the same temperature; the agar was poured deep; and all other necessary precautions were taken for accurate comparison. Daily measurements of colony diameter were made, and figure 4 shows the curves obtained for growth at different temperatures by plotting the measurements at the end of 5 days.

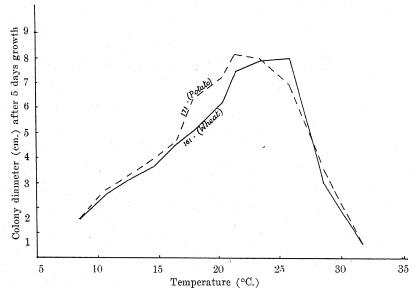


Fig. 4. Effect of temperature on the rate of growth of Rhizoctonia solani from wheat roots and from potatoes on potato-dextrose agar.

The cardinal temperatures for both fungi may be given as: maximum, 32° C.; optimum, 23–26° C.; minimum, 4° C. However, there was very little growth above 28° C., and below 13° C. the growth became extremely thin and spidery, scarcely visible to the naked eye.

(2) Growth on potato-dextrose agar adjusted to different reactions. The methods used in this experiment followed very closely those used by Webb and Fellows (11). Six isolates of the cereal Rhizoctonia and 2 isolates of Rhizoctonia solani from potatoes were compared. Measurements of colony diameter were made at the end of 3 days in order that the progressive

change towards acidity of the more alkaline agars should not affect the results too much.

The optimum reaction for the growth of the cereal Rhizoctonias lay between pH 6.0 and 6.5, while the optimum for the 2 isolates of *Rhizoctonia solani* from potatoes was perhaps slightly more alkaline, pH 6.5 to 7.0. However, the influence of reaction upon the rate of growth of the isolates from both hosts showed a good general correspondence over the whole range (Fig. 5). The separation of the curves in the vertical scale of this figure is a measure of the vigor of growth of different isolations only; the freshly isolated culture No. 183 from South Australian potatoes showed a very vigorous growth.

(3) Cross-inoculation tests. The cultures used were No. 161, Rhizoctonia from wheat, Tarcowie, South Australia, and No. 171, Rhizoctonia

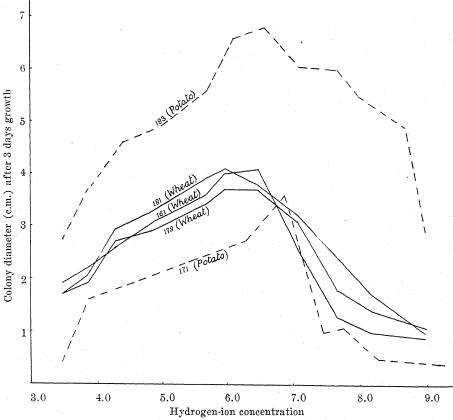


FIG. 5. Effect of hydrogen-ion concentration of the medium (potato-dextrose agar) on the rate of growth of 3 isolates of *Rhizoctonia solani* from wheat roots and 2 from potatoes.

solani from potatoes, New South Wales. The inoculum was multiplied on a mixture of sterilized oat and barley kernels, and lots of 10 gm. or 60 gm. of inoculum were used per 6-in. pot. Each fungus was tested on potatoes, wheat, oats, and barley, both in pure sand and in a sand-soil mixture.

After only 3 weeks' growth the inoculated pots were noticeably behind the controls. On washing out, the roots in all inoculated pots were found to be attacked, including cereals inoculated with the potato fungus and potatoes inoculated with the cereal fungus. The affected portions of the roots were browned, flaccid, and easily broken. Microscopical examination showed that the cortex was densely penetrated by the characteristic vigorous, refringent Rhizoctonia mycelium. Comparison of the severity of attack in the various pots showed that the cereal Rhizoctonia had affected the cereals somewhat more severely and that the potato Rhizoctonia had affected the potatoes more severely, as judged both from the number of points of attack on the roots and from the amount of mycelium in the cortex. The experiment demonstrated beyond question, however, that the Rhizoctonia from wheat would attack potatoes and that *Rhizoctonia solani* from potatoes would attack cereals. As mentioned above, this last point had been previously demonstrated by Rayllo (5).

(4) Effect of soil temperature on pathogenicity. Two experiments have been done in soil temperature tanks of the Wisconsin type and both showed that the cereal Rhizoctonia is a more vigorous parasite of wheat at low temperatures.

As an example of these experiments, Rhizoctonia No. 161 from wheat, Tarcowie, South Australia, was multiplied on oat-barley-kernel medium and 100 gm. of inoculum per 8-in. container was used in a soil-sand mixture, maintained at 50 per cent saturation by frequent weighings and addition of water at the correct temperature. Federation wheat was planted in the containers and the tanks were maintained at temperatures of 12, 17, 22, 27, and 32°.

The containers were washed out for examination of the roots 3 weeks after planting. The plants were most severely infected in the soil maintained at 12° C.; the majority of the roots were little more than browned stubs and the tops of the plants were correspondingly stunted. The plants were still fairly severely attacked at 17° C., but at 22° there was no perceptible effect on the tops and a much less severe root infection; at the higher temperatures the effect was very slight. Microscopic examination of the roots showed a relatively small amount of infection at the higher temperatures, and what mycelium there was in the cortex had none of the vigorous appearance characteristic of the infections at 12 and 17° C.

DISCUSSION

The isolations of Rhizoctonia from diseased patches in wheat and oat crops have agreed so closely in all characters tested with isolations of *Rhizoctonia solani* from potatoes that the cereal fungus may be definitely referred to this species.

In field inspections the very characteristic stunting effect of the fungus in the early seedling stage is liable to confusion with only one other disease in South Australia, namely, the nematode disease caused by *Heterodera schachtii* (1). The two diseases can be immediately differentiated by examining the roots, however. The nematode causes small but definite root-knots, with tufts of lateral roots arising from them, whereas plants affected by Rhizoetonia have the roots killed back to brown stumps.

The occurrence of the disease in the field during the seedling stage in winter corresponds with what one would expect from the soil-temperature-tank studies. The fungus is most active as a parasite at low temperatures, and seedlings that are not killed before spring usually recover in the warm weather. Generally similar temperature relations were obtained by Richards (6) in his study of the effect of *Rhizoctonia solani* on potatoes, and a number of other workers have also reported more vigorous parasitism on various plants at low to medium temperatures. However, definitely higher optimum temperatures for disease production have been reported by Walker (10) for this fungus on cotton and by Dickinson (2) for brown patch of turf.

It is curious that the fungus seems restricted entirely to the roots and is unable to attack the stems, even underground. This is in contrast to its effect on potatoes, cotton, and in brown patch of turf, and other diseases.

No sclerotia have been found in the field, although they were occasionally found on inoculated plants in pure sand. The fungus probably oversummers in the form of the thin, dark brown, distributive mycelium.

The fungus is scarcely of sufficient economic importance yet to call for definite experiments on control. Since the patches are still visible when the land is left out to grazing, owing to the disease affecting the pasture grasses and other plants, the leaving of the land to pasture is of no avail. The low rainfall in areas where the disease occurs practically prevents any crop other than cereals being grown in the rotation, and all the cereals are susceptible. Fallowing is the most hopeful method of control, but there have been a number of instances in which 1 year's fallow has not rid the soil of the fungus. The last 5 or 6 years in South Australia have been very dry, however, and it is possible that good working of the land in a year of normal rainfall will prove effective.

SUMMARY

Rhizoctonia solani is shown to be responsible for a definite seedling disease of wheat, oats, and barley on the low-rainfall, alkaline, "mallee" soils of South Australia.

The disease occurs usually in small, definite patches. The fungus attacks the roots, causing them to die back to brown stumps, and the affected seedlings remain stunted and later die. Pasture plants also are affected.

The disease is most active at low soil temperatures (12-18° C.).

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TRICHODERMA LIGNORUM AS A PARASITE OF OTHER SOIL FUNGI¹

R. WEINDLING

(Accepted for publication February 23, 1932)

The following observations regarding the parasitism of *Trichoderma lignorum* (Tode) Harz were made in connection with a study of the damping off of Citrus seedlings.² A fast-growing strain of *Rhizoctonia solani* Kühn³ was most frequently isolated from damped-off seedlings. In inoculation experiments it proved extremely virulent, especially on the recently germinated seedlings of sour orange, sweet orange, and grapefruit. *Phytophthora parasitica* Dastur and *Pythium* spp. also were isolated, but much less frequently than the first fungus. Moreover, these last two fungi appeared to be virulent only under certain favorable environmental conditions. Secondary invaders, repeatedly isolated from diseased roots, were *Fusarium* spp. and a strain of *Trichoderma lignorum*. By means of numerous inoculations both organisms were shown to be nonpathogenic to Citrus seedlings.

During these experiments Trichoderma hyphae were observed coiling around Rhizoctonia hyphae and destroying colonies of this fungus. The same result was obtained repeatedly on several culture media, the Petri dishes being inoculated simultaneously with pure cultures of both fungi.

In studying the peculiar parasitism of this fungus the following modes of attack were observed. Side branches of the advancing Trichoderma mycelium coil more or less tightly around aerial hyphae of Rhizoctonia or grow along them in straight or wavy lines. The protoplasm of the attacked hyphae coagulates and the cells lose their vacuolate structure (Fig. 1, A-D). Often the host hyphae break at a septum and some of the granular contents exude. Such a location seems to be particularly attractive for Trichoderma hyphae, which increase rapidly in number and diameter (Fig. 1, E, F; Fig. 2, A). Growth of the host hyphae stops almost abruptly with the initiation of the attack by the parasite. The more advanced the stage

¹ Paper No. 266, University of California, Graduate School of Tropical Agriculture and Citrus Experiment Station, Riverside, California.

² The investigation was made at the University of California, first, under the direction of J. T. Barrett, at Berkeley, later under the direction of H. S. Fawcett, at Riverside, who had originally suggested the damping-off problem. Valuable suggestions and help were also obtained from L. J. Klotz, T. E. Rawlins, and several other staff members of the Division of Plant Pathology at Berkeley and at Riverside.

³ For identification of the organisms, thanks are due to C. L. Shear, of Washington, D. C., and S. F. Ashby, of the Imperial Mycological Institute, Kew, England.

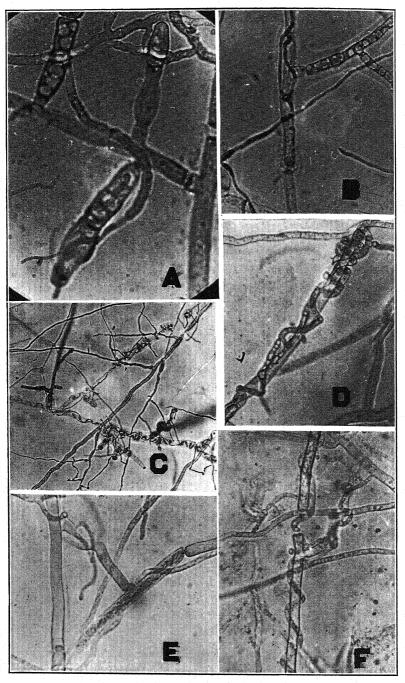


Fig. 1. A and B. Beginning of aerial attack on Rhizoctonia (citrus strain) by Trichoderma. Note stages in disappearance of vacuolate structure. The hyphae of smaller diameter are Trichoderma. A, \times 660; B, \times 330. C and D. Trichoderma coiling around host hyphae. C, \times 110; D, \times 330. E and F. Rhizoctonia hyphae breaking up at septa as the result of attack \times 330.

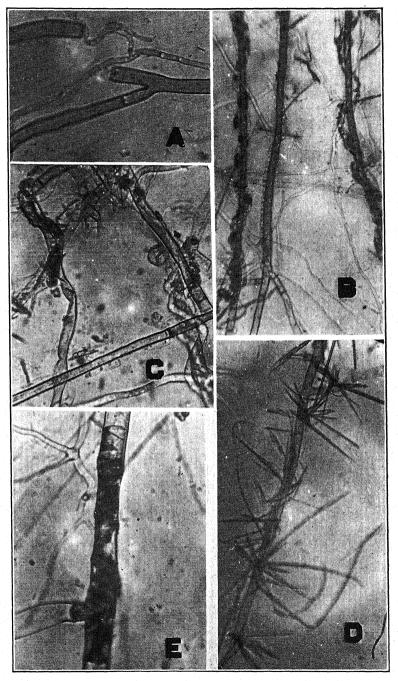


Fig. 2. A. Rhizoctonia (citrus strain) cells breaking up at septa as the result of aerial attack by Trichoderma. B to D. Autolysis stages of some of the attacking Trichoderma hyphae, showing the yellow protuberances that break up later into crystals. B, \times 190; C and D, \times 330. E, Rhizoctonia growing on the surface of medium, with Trichoderma close but not in contact. Host hypha becoming filled with dark yellow

of attack, the paler the host mycelium becomes and the less readily it stains with haematoxylin and other dyes. Frequently, coiling branches of the parasite form protuberances containing light-yellow granules. These protuberances finally break up, releasing numerous sheaves of yellow, needle-like crystals (Fig. 2, B-D). Most of the attacking hyphae, however, remain alive, and almost none of the thicker, nonattacking Trichoderma threads disintegrate. Also, living Rhizoctonia cells are occasionally found after all surrounding hyphae of this fungus seem to be killed.

While, with the "aerial attack" just described, the effect of the parasite on the host hyphae seems to be rather localized, this is much less the case below the surface of the medium. In the medium coiling occurs rarely, but, as the Trichoderma colony advances, host hyphae die and may break up in regions in advance of the invading parasite (Fig. 3, A). Darkyellowish masses accumulate in parts of some of the attacked hyphae, which later break up, forming masses of dark yellowish, star-like crystals (Fig. 3, B-E). The arrangement of these crystals frequently suggests explosive-like violence during the disintegration of the host hyphae. Colorless media appear aniline yellow (9) wherever the yellow pigment of hyphae and crystals is formed in large quantities. Occasionally, one may find a few Trichoderma segments becoming dark yellow and breaking up in the same way as the Rhizoctonia.

Also, in very old pure cultures of Trichoderma, sheaf-like, aerial crystals, as well as star-like, submerged ones, have been found, but always much less frequently than in the mixed cultures. In pure Rhizoctonia cultures, when killed by chloroform vapor, the hyphae lose their vacuolate structure in a manner similar to that shown during the first stage in Trichoderma attack. But they neither break at septa nor do they form yellow crystals.

While external parasitic attack is prevalent, internal parasitism seemed to occur in some hyphae of a strain of *Rhizoctonia solani* isolated by H. S. Fawcett from potatoes (Fig. 3, F, G). The latter fungus does not form true anastomoses with the Rhizoctonia strain from Citrus. Moreover, the strain from potato is essentially nonparasitic on Citrus seedlings and appears to be more susceptible to the attack of Trichoderma, especially in water-agar media.

With Pythium spp. and Phytophthora spp., grown in conjunction with Trichoderma, similar star-like and sheaf-like crystals were formed from disintegrating hyphae (Fig. 4, A). In general, these fungi seem to be more readily overcome by the parasite than does the Rhizoctonia. Other fungi found up to now that are more or less susceptible to the attack of Trichoderma are: Sclerotium rolfsii Sacc. (Fig. 4, B) and Rhizopus spp. (Fig. 4, C). Several variations in the mode of parasitism have been observed on

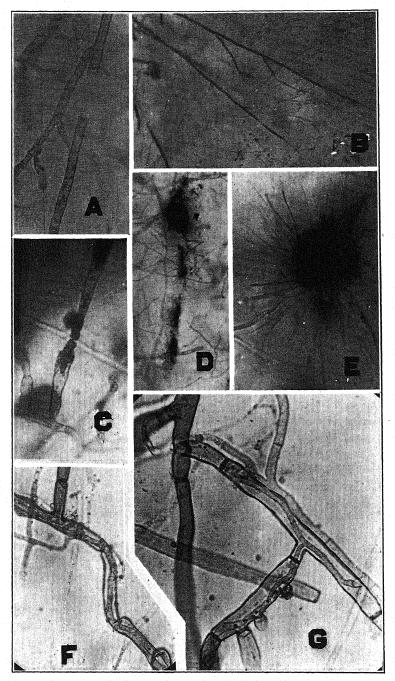


Fig. 3. A-E. Submerged attack of Rhizoctonia (citrus strain) by Trichoderma. A. Dead Rhizoctonia hypha breaking up at a septum and releasing granular contents. \times 300. B-E. Stages in the disintegration of host hyphae. B, \times 65; C, \times 330; D, \times 160; E, \times 450. F and G. Internal parasitism of Trichoderma in *Rhizoctonia solani* (strain from potato). Note connections of coiling hypha with internal hypha in figure F. F. \times 400: G. \times 600.

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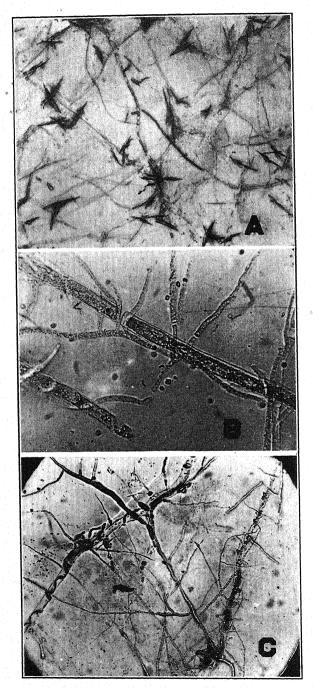


Fig. 4. A. Stages in the disintegration of Pythium hyphae when attacked by Trichoderma. Note sheaves of crystals on the surface of the culture medium. × 80. B. Initial attack of Trichoderma on Sclerotium rolfsii showing one break at a septum.

hosts other than Rhizoctonia and on this fungus when grown on different media or attacked in later stages of growth. It is beyond the scope of this preliminary paper to describe these modifications. Essentially, the manner of the Trichoderma attack seems to be similar to that outlined.

Most of the material for the pictures was obtained by removing small areas of glucose-agar plate cultures to glass slides. The cultures had been incubated at 21 or 28° C. in a moist atmosphere. Slight drying-out of the media stops the growth of Trichoderma. Some material was grown on agar films on cover slips that were sealed to glass slides; some of the material was not stained, and some stained with vital stains (Congo red 1:80,000).

It is conceivable that in some cases Trichoderma may act merely as a competitor for food, while incidentally killing other fungi in mixed cultures. The aerial attack, however, suggests strongly that substances from the host hyphae are utilized as nutrients. Moreover, while the fructification of the parasitic fungus is usually delayed where opposed colonies meet, the mycelium as well as the fructification of Trichoderma seems eventually to be more abundant in mixed than in pure cultures.

That parasitism, independent of any medium, may take place whenever the parasitic fungus is actively growing and has sufficient moisture, was demonstrated by the following procedure. Rhizoctonia hyphae were permitted to grow out of pure cultures onto sterile slides that did not touch the medium in the Petri dish. Similarly, Trichoderma hyphae were obtained on cover slips free from media. The inverted cover slips were then sealed to the slides, leaving a small air space between them and allowing the entrance of moist air through openings in the sealing. On these slides, which were kept in moist Petri dishes, branch hyphae of Trichoderma growing along the host filaments were observed to acquire a much larger diameter in an equal period than the main hyphae from which they originated.

The writer has not yet determined whether enzymes or toxins are the active principles in this parasitic action. Apparently, to be effective in aerial attack, these substances must be brought into close contact with the host hyphae, since there is no medium immediately present for diffusion of these products. This localization of attack seems to bring about destruction of both the fungus host and parts of the parasite, itself, the attacking hyphae of the latter eventually being autolyzed. When the attack is within the medium, however, the destructive products appear to diffuse ahead, killing the host hyphae and having little autolytic effect on the parasite. On the surface of the medium, there is a gradation of these two distinct modes of attack. Hyphae filled with yellow material have been observed at that location, probably giving rise to aerial, sheaf-like crystals (Fig. 2, E). This suggests the possibility that the sheaves of aerial crys-

tals and the submerged, star-shape crystals may be chemically the same, their difference in physical appearance being effected by the surrounding medium. In the chemical tests that follow, the medium may also have had some influence on the results. The aerial crystals were found to be soluble in chloroform. The submerged yellow hyphae and crystals gave a deep red color with 20 per cent KOH. This reaction may be due to the presence of a pigment similar to that described for certain Penicillia and Aspergilli (2).

Antagonistic effects among microorganisms have been frequently observed, and the literature on this subject has been ably reviewed elsewhere (4). Parasitism among fungi has been reported for many organisms (12), but, excepting in the Mucorales, it has not often been described in detail (1, 8). The perfect stage of Trichoderma lignorum is said to be a species of the genus Hypocrea. Other species of this genus have been reported as parasites on fructifications of many Basidiomycetes (10, 12). In the inoculation of soil supporting beet and pine seedlings, it was shown (3, 5) that parasitic and saprophytic fungi together caused less loss than parasitic fungi alone. Attempts have been made to control parasitic organisms by their antibiotic enemies (6, 7).

The research reported in this paper suggests that under certain conditions Trichoderma might be used for the biological control of fungus diseases. For example, in damping off, in which the most susceptible period of certain seedlings is relatively short, an abundant inoculation of the soil with the protective organism may tide them through the critical period, before the plant parasites have become reestablished. It will probably be difficult to keep Trichoderma dominant in the surface soil because of its tendency to self-digestion and because the vegetative form of the fungus is dependent on continuous moisture. It is reported to be a fungus par excellence of water-logged soils (11); that is, it is favored by conditions good for most parasitic fungi and bad for most plants. Preliminary pot experiments with Citrus seedlings have revealed certain difficulties but have shown some striking cases of the protective action of Trichoderma in preventing damping off. These data as well as others that are being collected will be reserved for later publication.

SUMMARY

In the course of an investigation of damping off of Citrus seedlings, a strain of *Trichoderma lignorum* has been found to parasitize a number of pathogenic soil fungi in cultures, *i.e.*, *Rhizoctonia solani*, *Phytophthora parasitica*, *Pythium* spp., *Rhizopus* spp., and *Sclerotium rolfsii*.

The inhibition and death of the host hyphae were brought about in two ways: (a) in aerial hyphae by close contact or by coiling of Trichoderma

around them; (b) in submerged hyphae by action at a little distance more frequently than by contact.

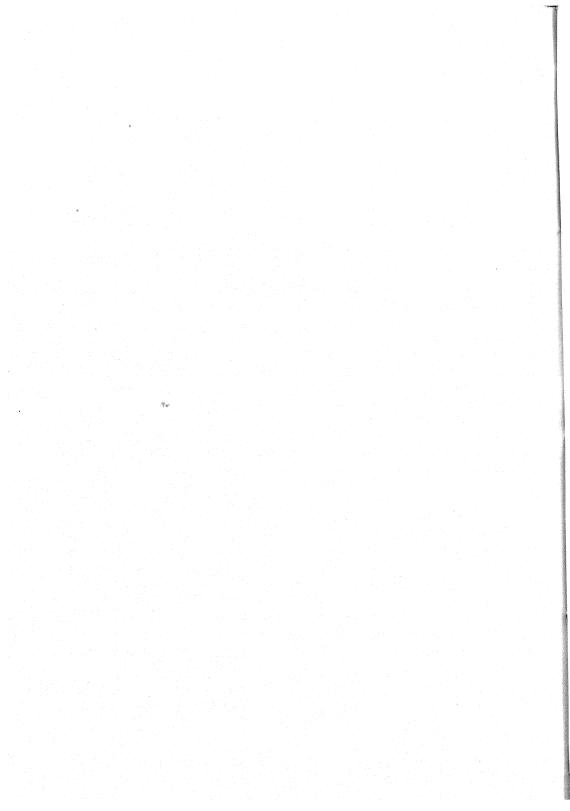
Trichoderma hyphae were also found to attack Rhizoctonia hyphae, when both fungi were free from any connection with nutrient media.

Based on preliminary pot experiments, the possibility is suggested of controlling certain pathogenic soil organisms by abundant inoculation of the soil with cultures of *Trichoderma lignorum*.

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REACTION OF MARTIN WHEAT TO THREE PHYSIOLOGIC FORMS OF TILLETIA TRITICI¹

WILLIAM K. SMITH (Accepted for publication February 8, 1932)

Within Tilletia tritici (Bjerk.) Wint. and T. levis Kühn, the 2 species of fungi causing stinking smut of wheat, the presence of numerous physiologic forms has now been demonstrated. Practically all determinations have been made on the basis of the percentage of bunt on differential varieties of wheat inoculated with different collections. However, in some preliminary trials with a large number of collections, Bressman² observed indications of differences between some collections in the texture of the smut ball, in the occurrence of masses of bunt spores on the outside of the glumes, and in the optimum temperature for spore germination. Rodenhiser³ found a significant difference between 2 physiologic forms of T. tritici in the stunting of the bunted culms of Little Club.

From the collections of bunt reported by Gaines⁴ in 1928, three physiologic forms of Tilletia tritici were later distinguished by the reaction of a number of differential varieties of wheat. As was pointed out in another paper,⁵ one physiologic form, T2, could readily be recognized as distinct from T1 and T3 by the percentage of bunt on various differential varieties but particularly by the reaction of Martin (C. I. 4463), a variety of Triticum vulgare Vill. In a rod-row test sown in the fall of 1928, Martin was bunt-free when inoculated with T1, gave 19 per cent of bunted heads with T2, and 71 per cent with T3, while Hybrid 128 (C. I. 4512) was uniformly susceptible to all forms. In later trials, the reaction of Martin has approximated that observed in 1929. T2, however, can also be differentiated from T3 by the appearance of the bunted spikes of Martin. Because the spikes of this variety, invaded by T3, are indistinguishable from those invaded by any of the physiologic forms of T. tritici other than T2 or of T. levis in tests at the Washington Agricultural Experiment Station, it is apparent that T2 can be distinguished from any of these

¹ Published as Scientific Paper No. 218, College of Agriculture and Experiment Station, State College of Washington.

² Bressman, E. N. Varietal resistance, physiologic specialization, and inheritance studies in bunt of wheat. Oregon Agr. Exp. Sta. Bul. 281. 1931.

³ Rodenhiser, H. A. Stunting of wheat caused by *Tilletia levis* and *T. tritici*. Jour. Agr. Res. 43: 465-468. 1931.

⁴ Gaines, E. F. New physiologic forms of *Tilletia levis* and *T. tritici*. Phytopath. 18: 579-588. 1928.

⁵ Smith, W. K. Inheritance of reaction of wheat to physiologic forms of *Tilletia levis* and *T. tritici*. Jour. Agr. Res. In press.

forms by the reaction of Martin. Barrus⁶ has called attention to the significant differences between healthy and bunted culms in a white variety of *T. vulgare* similar to Dawson's Golden Chaff, and approximately the same differences are apparent in Martin between bunt-free culms and culms invaded by T3; it is not necessary, therefore, to enumerate these differences. A comparison will, however, be made between culms of Martin smutted with T2 and culms smutted with T3. Material adequate for this comparison was grown from seeds planted in adjacent rows in the fall of 1930, each row being inoculated with one of the three physiologic forms.

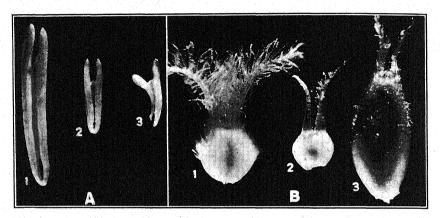


Fig. 1. A. Anthers of Martin wheat: 1, from bunt-free flower; 2, from flower infected with physiologic form T2; 3, from flower infected with T3. B. Ovaries of Martin wheat: 1, bunt-free; 2, infected with T2; 3, infected with T3.

The first observations were made on diseased culms when the spike had partly emerged from the uppermost leaf sheath. At this stage of growth the following characteristics were noted: (1) On T2 culms, i.e., culms of which the spike was invaded by physiologic form T2, the length (4 to 12 cm.) and width (3 to 6 mm.) of the uppermost leaf were considerably less than the length (15 to 24 cm.) and width (10 to 15 mm.) of the corresponding leaf on healthy and on T3 culms; moreover, the T2 leaf was markedly twisted. (2) The length of the anthers (2.2 to 3.2 mm.) in the flowers of the T2 spike was somewhat greater than that in the flowers of the T3 spike (1.3 to 1.9 mm.); the anthers in the T2 flowers were less distorted and were greener than those in the T3 flowers (Fig. 1, A). (3) The length of the ovary (1 to 1.8 mm.) on T2 spikes was on the average slightly less than that in bunt-free spikes, while the ovary in each was approximately of the

⁶ Barrus, M. F. Observations on the pathological morphology of stinking smut of wheat. Phytopath. 6: 21-28, 1916.

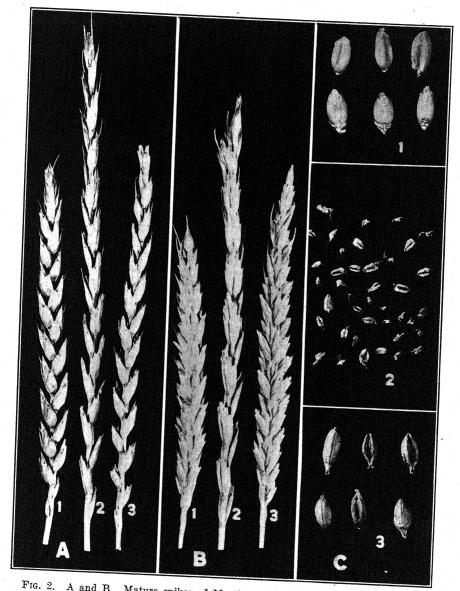


Fig. 2. A and B. Mature spikes of Martin wheat: 1, bunt-free; 2, infected with T2; 3, infected with T3. C. 1, bunt-free kernels; 2, bunt balls from infection by T2; 3. bunt balls from infection by T3.

same color—white to pale green (Fig. 1, B); these contrasted sharply with the dark-green and swollen ovary (2.2 to 3.7 mm. in length) of the T3 spikes. The stigmas in the T2 and T3 heads were poorly developed in comparison with those in bunt-free plants.

At maturity the following differences are apparent: (1) Spikes infected with T2 (Fig. 2, A and B) are more lax than those infected with T3; from measurements made on heads having a comparable number of spikelets per head, the average length of 10 internodes in the middle of the head for the T2 group was 6.93 ± .08 cm., while that for the T3 group was $5.72 \pm .04$ cm., giving a significant difference of $1.21 \pm .09$ cm. (2) The T2 spikes are much narrower than the normally bunted spikes in which the smut balls protrude from the glumes; the T2 spikes resemble sterile spikes in appearance. This condition is due to the fact that the smut balls arising from the infection of Martin by T2, while having a considerable range in size, are very small (0.5 to 3.5 mm, in length) in comparison with smut balls arising from infection by T3 (4.5 to 6.5 mm.); the T2 smut balls are shrivelled and angular (Fig. 2, C). The spores in the abnormal smut balls seem to be in various stages of development; in some balls, particularly the larger ones, a proportion of spherical, thick-wall spores with welldefined reticulations has been observed, but there is present also a large proportion of thin-wall, apparently immature spores that are often much distorted in shape.

The reaction of Martin to T2 is a type of resistance; yet it is a resistance that is of practical value only in reducing the amount of inoculum, because, although ordinary smut balls do not develop, healthy kernels are not produced. The condition, however, is of interest in being a character by which this physiologic form may be distinguished from other physiologic forms of bunt.

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LEAF TEMPERATURE OF LETTUCE AND ITS RELATION TO TIPBURN

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(Accepted for publication February 23, 1932)

The symptoms of tipburn of lettuce were first described in 1891 by Jones (1), but he did not mention causal agency. Since that time many explanations of the cause of this disease have been offered. Although organisms have been found in tipburn tissue, there is no evidence that they cause the disease. Superabundant soil moisture and excessive application of fertilizers have been considered responsible for tipburn, and there is also a general impression that the injury is due to high temperature and rapid transpiration.

The relation of leaf temperature during the process of tipburning of lettuce has received very little attention. In order to obtain some information on this question, investigations were undertaken to study the temperature of (1) leaves during day and night, (2) various portions of leaves under identical conditions, and (3) leaves in direct and diffuse light.

A brief report of the results of these investigations was made in 1927 (2).

METHODS OF EXPERIMENTATION

The leaf temperatures in these experiments were determined by means of thermocouples pressed against the surface of the leaf, as suggested by Shreve (4). A Leeds and Northrup No. 2500-A galvanometer was used to make the determinations. In all experiments the cold junction was kept in a thermos bottle containing distilled water. The other junction was clamped upon the surface of the leaf, care being taken to make certain of a good contact. Air temperatures were always determined by a standardized thermometer, graduated to tenths of a degree and shaded from the sun but near the leaf under investigation.

The temperature determinations were made on lettuce plants grown in a greenhouse. Certain leaves were selected for a given series of determinations, care always being taken to select leaves of about the same age and with approximately the same exposure to the incident rays of the sun. In a number of instances temperature determinations were made of leaves that were in the process of tipburning.

¹ Formerly Assistant Plant Pathologist, Colorado Agricultural Experiment Station. The data reported herein were procured while the writer was a member of its staff.

² The writer gratefully acknowledges the helpful criticism of Drs. L. W. Durrell and E. C. Stakman in the preparation of this manuscript.

EXPERIMENTAL DATA

The temperature of an intact leaf or a plant exposed to atmospheric conditions is influenced by many factors, the most important of which are air temperature, air currents, intensity of light to which the plant is exposed, and relative atmospheric humidity. In these data only the relation of leaf temperature to air temperature has been considered in regard to tipburn of lettuce.

Temperature of different portions of the same leaf. Some determinations were made to compare the temperature of base and tip of leaves in both sun and diffuse light, the results of which are shown in table 1. The

TABLE 1.—Average leaf temperatures of upper and lower surfaces of healthy lettuce leaves in direct and diffuse light

	Average temperature									
Number of		Base of leaf	Tip of leaf							
determinations	Air	Upper Lower surface surface	Upper surface	Lower surface						
	°C.	°C. °C.	°C.	°C.						
	4.	In direct sunlight								
34	22.6	20.6 20.5	21.3	21.2						
		In diffuse light								
34	19.0	20.6 20.3	21.3	21.1						

conditions necessary to produce diffuse light were obtained by moving plants in the shade of an 18-in. by 30-in. round galvanized can upon which the galvanometer was resting.

The data presented in table 1 show that, in the sun, the temperature of any portion of the leaves is always consistently lower than the temperature of the surrounding air. The leaf temperature in the shade, however, is just the reverse, being higher than the air temperature. For example, the average temperature of the top of the base in the sun is 2° C. lower than the air temperature, while this portion of the leaf in the shade is 1.6° C. higher than the surrounding air temperature.

Miller and Saunders (3) found that the temperature of leaves was higher than the air temperature in the sun but was cooler than the surrounding atmosphere in diffuse light. This latter statement is the reverse of the results obtained in the experiments here reported.

From the data in table 1 it is evident that the top surface of the leaf is slightly warmer than the corresponding portion on the lower surface. In the sun the top of the base of the leaves had a temperature of 20.6° C., while the lower surface was 20.5° C. Likewise, in the shade, the top of the

TABLE 2.—Average temperature of lettuce leaves obtained under conditions favorable for tipburn

Temperature of lower surface of tip		22.6	22.1	22.7	23.3	25.0	25.3	23.0	24.1	26.2
Temperature of upper surface of tip	°C.	22.5	21.9	22.4	22.4	22.0	22.1	23.1	24.5	26.4
Temperature of lower surface of base	°C.	22.0	21.8	22.0	24.0	25.1	24.9	23.4	23.5	25.8
Temperature of upper surface of base	့်	22.1	22.1	21.8	23.6	25.2	25.0	23.2	23.6	25.9
Temperature of air	°C.	23.0	23.3	25.5	28.6	29.2	29.3	34.9	31.6	32.6
Number of observations		9	21	6	9	9	9	9	9	9
Time of observation		4:30 p. m.	9:15 p. m.	12: 00 m.	3:05 a. m.	4:15 a. m.	5:15 a. m.	6:10 a. m.	7:05 a. m.	8:00 a. m.

base was 20.6° C., or 0.3° C. higher than the lower surface, while the upper surface of the tip was 0.2° C. higher than the lower surface. These data correspond with those of Miller and Saunders (3), who also found that the temperature of the upper surface of the leaf is higher than that of the lower surface in both sun and diffuse light.

The temperature of the tip is higher than that of the base of the same leaf in both sun and diffuse light (table 1). In the sun the top of the tip is 0.7° C. and the lower surface 0.7° C. warmer than the respective sides of the base. Similarly, in the shade, the temperature of the upper surface of the tip is 0.7° C. and the lower surface is 0.8° C. higher than the temperature of the upper and lower surfaces of the base. These observations are similar to those of Miller and Saunders (3), who showed that the temperature of the base of leaves is always lower than that of the tip in direct sunlight.

Leaf temperatures during the night. On the night of March 8, 1926, and the morning of March 9, 1926, readings were made on lettuce plants that were in glass chambers in the greenhouse and the plants under favorable conditions for tipburn to occur. All the plants used in these experiments became tipburned between 9:15 p.m. and 3:00 a.m.

From the data in table 2 it is evident that the temperature of the leaves was considerably lower than that of the air throughout the duration of the experiment. The temperature of the tip and of the base of the leaves fluctuated considerably during this experiment, making a correlation impossible. The important point in these data is that tipburn occurred, although the leaf temperature of these plants was consistently below the air temperature.

Since some of the plants tipburned between 9:15 p.m. and 12:00 m. when the temperature of the tips was 1.2° C. and 3.7° C. below the air temperature at these hours, it appears that tipburn is not caused by excess low temperature.

DISCUSSION

On normal healthy lettuce plants in sunlight the upper surface of the base of the leaves was found to be 2.0° C. and the lower surface 2.1° C. cooler than the surrounding air temperature. In diffuse light, the upper surface of the base was 1.6° C. and the lower surface 1.3° C. warmer than the temperature of the air.

The edge of healthy leaves was found to be only 0.7° C. to 0.8° C. warmer than the base of the leaf.

Hourly temperature readings, made during the night on lettuce leaves during the process of tipburning, showed that the upper surface of the tip ranged from 0.5° C. to 11.8° C. lower than the air temperature. During

the time that tipburning was rapidly occurring (9:15 p. m. to 12:00 m.) the range was from 0.5° C. to 3.1° C. below the temperature of the surrounding air. It therefore is evident, from the data presented, that tipburn of lettuce is not caused by an excessive temperature of the leaf tissues.

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STUDIES ON BORDEAUX DEPOSITION

GEO. L. HOCKENYOS AND GEO. R. IRWIN (Accepted for publication February 1, 1932)

The study of supplements for Bordeaux spray can hardly be called a neglected one. Holland, Dunbar, and Gilligan, in Massachusetts, give an excellent review of the literature on this subject. They list 97 references and call attention to the fact that much of the evidence is contradictory. The present work is an attempt by laboratory studies to answer these questions: What effect does the use of a wetting agent have on the uniformity of the deposit on the leaf surface and what effect does it have on the total deposit per unit area?

The apparatus used in spraying the leaves consisted of a Hudson hand sprayer operated by 10-lbs. air pressure. The spray was directed against a perpendicular baffle board at a fixed distance from the tip of the nozzle. The spray was cut off close to the nozzle by a swinging shutter that was attached to a weighted pendulum in such a position that, when the shutter was in place to cut off the spray, the pendulum would be raised 45°. The plane of the arc described by the shutter-pendulum system was at right angles to the direction of the spray jet and parallel to the surface being sprayed. In operation, the spray jet would be directed against the perpendicular board and a point directly in the center of the area of deposition noted. The shutter would now be swung into position to cut off the spray and so held until the leaf to be sprayed was fastened to the baffle. A sheet of absorbent paper was used to keep the unsprayed side of the leaf clean. When the shutter was released, the pendulum-shutter system would swing through a long arc and, by adjusting the length of the pendulum arm, the time of each full period was made equal to 2 seconds. Thus, each swing of the pendulum represented 2 seconds of time that the spray was directed against the leaf. It was found that the shutter would return near enough to its original position to cut off the spray for 5 consecutive swings.

After spraying, the leaves were dried and sections cut from them with a cork borer 0.26 in in diameter. These sections were analyzed for copper by the method described by Hockenyos in a previous publication.² In the tests where studies of uniformity of deposit were made, 9 sections were taken from each leaf according to a definite pattern and both the average deposit and the percentage irregularity calculated. The latter computa-

¹ Holland, E. B., C. O. Dunbar, and G. M. Gilligan. Supplements for copper fungicides. Massachusetts Agr. Exp. Sta. Bul. 252: 94-112. 1929.

² Hockenyos, George L. Solubility of Bordeaux. Phytopath. 21: 231-234. 1931.

tion was made by finding the average of the deviation from the average deposit per section and dividing by the average deposit.

TABLE 1—Showing how deposit of copper increases with time of spraying where 4-4-50 Bordeaux is used without a supplement. Uniform cherry leaves were used and sprayed on the upper surface at a distance of 13 in. from the nozzle

Time in seconds	Copper per sq. in.	Percentage irregularity
12	.00011 gr.	10
16	.00020	18
20	.00015	38
24	.00010	16

In this test, it was noted that run-off began at 20 seconds, and that fact doubtless accounts for the drop-off in total deposit and the increase in irregularity at this point. It is interesting to note that according to Holland, Dunbar, and Gilligan,³ Guba analyzed cucumber leaves for the amount of copper per sq. in. and found approximately 0.00009 gr. Considering the differences in method of spraying, nature of leaf surface, and methods of analysis, this may be regarded as good correspondence.

In a second test, apple leaves were sprayed and analyzed by the same method as in the above test, except that the leaves were only 11 in. from the tip of the spray nozzle. It was noted that run-off began in 6 seconds.

TABLE 2.—Number of copper deposits and their analysis when blood is used as wetting agent

Time— seconds	and the first of t		Heavy spots	Irregularity		
	Per ct.			Per ct.		
6	None	0.00009	None	29		
6	"	0.00010	One (0.00066)	12		
6	"	0.00010	Two (0.00038)	20		
			(0.00016)			
6	1/25	0.00011	None	10		
6	1/25	0.00009	"	26		
8	None	0.00010	c.c	34		
8	"	0.00010	One (0.00050)	21		
8	1/25	0.00010	None	14		
8	1/25	0.00010		19		

³ Loc. cit., page 105.

Bordeaux 4–4–50 alone was compared with Bordeaux 4–4–50 plus 1/25 per cent dried-blood albumin. The leaves were examined for heavy local deposits or spots, and, where such occurred, they were sectioned out separately and analyzed.

An examination of this table shows that the addition of blood albumin as a wetting agent did not decrease the amount of copper deposited but did eliminate the formation of heavy spots and to some extent increased the uniformity of the deposit.

From this table we see that the addition agents have had little effect on the amount of copper deposited and that the under surface of the leaf is capable of holding roughly twice as much copper as the upper surface,

TABLE 3.—A comparison of the amount of copper deposited per sq. in. on peach leaves.

Eight-second spraying time at 11 in. gave considerable run-off in all cases but did

not insure uniform wetting. Bordeaux 4-4-50 was used in all cases

Time—seconds	Supplement	Leaf surface	Gr. copper per sq. in
	Per ct.		
8	None	Upper	0.000078
"	"		0.000081
"		"	0.000078
		"	0.000090
	"	"	0.000090
10	ϵ	"	0.000111
"	••	Lower	0.000204
8	1 Colloidal clay	Upper	0.000075
"	10 p. 10 10 10 10 10 10 10 10 10 10 10 10 10	44	0.000075
	er to the second	66	0.000087
"	**	66	0.000111
"	1/2 Soap	66	0.000090
"	• •	"	0.000090
			0.000084
"	**	46	0.000096
"	1/8 Kayso		0.000096
"			0.000090
"	100 miles (100 miles 100 miles	"	0.000096
	1/8 Gum ghetti	66	0.000066
"	"	"	0.000098
"	1/8 Waste pulp liquor		0.000090
"	(Parp 114	"	0.000084
"	1/20 Blood albumin		0.000075
"	1/20 23004 4334		0.000090
1 46 m		66	0.000090
"			0.000093
"			0.000093
"		Lower	0.00033

whether or not a wetting agent is used. The wetting ability of each of the supplements used in table 3 was tested by noting with a stop-watch the number of seconds to give complete wetting of the entire leaf surface at a distance of 11 in. from the spray nozzle. The under surface of peach leaves was used and 6 leaves successively sprayed with each material.

TABLE 4.—Showing wetting ability of the several supplements indicated in table 3, expressed in number of seconds required to completely wet entire leaf surface at a distance of 11 in. from spray nozzle

Check—	Bordeaux a	one	5,	45,	8,	8,	8,	10	seconds
Bordeaux plus	1/20 per cer	t Blood albumin	6,	6,	6,	7,	6,	7	"
	1/8 " "	Kayso	8,	9,	5,	9,	5,	7	
ii ii	1/8 " "	Gum ghetti	8	12,	7,	14,	13,	25	
i ii ii ii	1/8 " "	Pulp liquor	8	8,	15,	10,	7,	10	" "
" "	1/2 " "	Soap	4	4,	5,	5,	4,	5	"
	1 " "	Colloidal clay	15	, 15,	15,	20,	13,	20	

That a great variation exists in the resistance of leaves chosen for their apparent uniformity is readily seen. The soap, blood albumin, and Kayso are shown to greatly enhance wetting, but it will be noted in table 3 that the average deposit of copper is as heavy as when no supplement was used at all.

From the above data, it seems a reasonable conclusion that the addition of a wetting agent to Bordeaux will, to a certain extent, increase the uniformity of the deposit, will practically eliminate the deposition of heavy spots, and will not decrease the amount of copper deposited per unit area.

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FUNGICIDAL VALUE OF PINE-TAR OIL AND COPPER RESINATE

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(Accepted for publication March 2, 1932)

The use of petroleum oil as an insecticide has proven of great value to the entomologists in the control of orchard and garden insects. A serious criticism of this spray, however, has been its lack of fungicidal value, frequently necessitating two spray applications or attempts at tank combinations, such as Bordeaux and petroleum oil, which usually leads to a distinct lowering of the insecticidal value of the oil. This has been noted in the writer's experiments² and confirmed in a later paper.³ The following typical examples of the need for combined oil-fungicidal sprays will serve to illustrate this:

Navel rot in oranges, especially prevalent in oil-sprayed citrus groves; a dormant spray for peaches in the warmer districts where lime-sulphur injury frequently results from dormant application. The delayed dormant spray for apples where residual sulphur from lime-sulphur application leads to oil-sulphur injury in the early summer.

Research covering a period of 4 years has developed a pine-tar oil that, through factory treatment, is prepared specifically for use as a spray on plants. This special fraction has specifications rather similar to a number of other oils derived from the pine tree by destructive distillation and the use of steam, so, for convenience in designation, this fraction is known as "Palustrex," which name will be used throughout this paper. Palustrex is in itself a more active fungicide than the petroleum fractions used for foliage spraying, and, in addition to this property, it is an active solvent for copper resinate. Such combinations of oil and copper have the physical advantages of an oil spray that are not possessed by a water-carried application, the oil emulsion having a lower surface tension, being active as a wax solvent and capable of greater penetration than is possible with water-carried sprays, such as Bordeaux. It should be noted that this is a true solution of copper in oil and not simply a suspension, consequently

¹ Consulting Entomologist, San Francisco, California.

² Ong, E. R. de, Hugh Knight, and J. C. Chamberlin. A preliminary study of petroleum oil as an insecticide for citrus trees. Hilgardia 2: 351-384. 1927. p. 362.

³ Porter, B. A., and R. F. Sazama. Influence of Bordeaux mixture on the efficiency of lubricating-oil emulsions in the control of San Jose scale. Jour. Agr. Res. 40: 755-766. 1930.

⁴ Woglum, R. S., et al. Handbook of citrus insect control for 1930. California Fruit Growers' Exch., Los Angeles, Bul. 7: 1930. pp. 2-3.

the copper is carried in minute amounts wherever the oil penetrates, and for this reason lower concentrations of copper should be used than when applied as a suspension in water. Besides the fungicidal value, the antiseptic value of Palustrex as a preservative for oil emulsions made with organic emulsifiers has also been established as slightly superior to cresylic acid.⁵

Palustrex may be used alone in a water-soluble form both as a fungicide and as an insecticide for susceptible insects, such as aphids. For resistant forms, it becomes necessary to combine Palustrex with a suitable petroleum fraction to increase the viscosity and raise the evaporation point. Copper resinate and nicotine, if desired, may also be added. The wax solvency of such blends is improved by the addition of Palustrex, as shown by the following data: 98 per cent of a 0.3 gm. sample of apple wax dissolved in 3 cc. of Palustrex, the time required for complete solution being 2 minutes, the larger part dissolving almost instantly on stirring into the oil; 72 per cent of a similar 0.3 gm. sample of wax dissolved in 22 cc. of kerosene after 15 minutes' exposure; and 7 per cent of a similar wax sample dissolved in 19 cc. of lubricating oil after a period of 1 hour. In a test with beeswax it was found that a saturated solution of Palustrex contained 10 times as much wax as did kerosene.

Field tests of the copper-oil sprays have confirmed laboratory findings in that walnut blight has been checked to a greater extent with the dual-purpose sprays than was accomplished by standard Bordeaux. Similar results have also been noted in the mildew (*Erysiphe cichoracearum DC*.) attacking canteloupes, where the soluble Palustrex and copper proved more efficient than the standard Bordeaux spray. Extensive field tests are being made this winter with such oil-copper combination in the control of peach leaf curl and blight, brown rot, and other diseases.

The comparative toxicity of copper resinate and copper sulphate is indicated in the results shown in table 1. Such comparisons between copper resinate and copper sulphate cannot be made in an exact way since one is water-soluble and the other oil-soluble; consequently, the data does not represent the full value of the use of an oil-soluble material nor does it give opportunity for showing the results of superior physical values, including lower surface tension, wax solvency, etc. It may be noted, however, that for equal weights of the 2 copper salts the copper resinate gave almost equal value, although, as stated, in water suspensions the full physical values are not brought out nor does this take into consideration the variation in content of metallic copper.

Since copper resinate has not been in general use, the following brief discussion will be given regarding the comparative status of this salt and

⁵ Ong, E. R. de. Present trend of oil sprays. Jour. Econ. Ent. 24: 978-985. 1931.

TABLE 1.—Spore tests of the comparative toxicity of copper resinate and copper sulphate

Toxic agent	Percentage of metallic copper	Spores	Spores germinated	Percentage germinated	
Monilia					
Check 10 tests in distilled water		260	192	73.90	
20 tests with copper sulphate	25.4	557	20	3.59	
20 tests with copper resinate	9.5	433	30	6.92	
Botrytis					
Check 10 tests in distilled water		235	147	62.50	
20 tests with copper sulphate	25.4	529	25	4.73	
20 tests with copper resinate	9.5	539	37	7.30	

the more common copper sulphate. Copper sulphate has the following formula: $\mathrm{CuSO_4} \cdot 5\mathrm{H_2O}$; it contains 25.4 per cent metallic copper and 36 per cent moisture. Copper resinate has the following formula: $\mathrm{Cu}(\mathrm{C_{20}H_{29}O_2})_2$; contains 9.5 per cent metallic copper and no moisture. The lower content of metallic copper in the resinate salt is an advantage from the standpoint of metallic residues on both fruit and vegetables, which overcomes the criticism sometimes made in the past of too free use of Bordeaux. Copper resinate is very light and fluffy and somewhat sticky in nature from the high content of resin present. The physical nature of the salt makes it difficult to mix with oil, so the same companies that market the specially prepared pine-tar oil known as Palustrex are now putting out a paste containing 70 per cent copper resinate dissolved in 30 per cent Palustrex. This is comparable to lead ground in oil and obviates the mechanical difficulty of mixing and also the danger to the operator of working with a light copper dust that might be inhaled in excessive quantities.

Field tests of blends of Palustrex, petroleum and copper resinate are being made as follows: 16 gal. of Palustrex, containing 25 per cent by weight of copper resinate, are added to 80 gal. of petroleum oil. This blend is then made up into an emulsion of 122 gallons. Using 5 gal. of this emulsion to 100 gal. of spray would give 0.158 per cent anhydrous copper resinate in the dilute spray. In a Bordeaux preparation containing 8 lb. of copper sulphate to 100 gal., there would be 0.65 per cent anhydrous copper sulphate in the dilute spray. The above formulas are for dormant spraying. These proportions are, of course, subject to further adjustment, particularly for specific purposes for which extensive work is planned on broad lines.

SUMMARY

A discussion of the present weakness of petroleum-oil sprays due to their lack of fungicidal value. The use of pine-tar oil, specially treated to promote safety on plants, as a general spray material and as a solvent for other toxic chemicals. The combination of the oil-soluble copper resinate with the treated pine-tar oil to give both insecticidal and fungicidal action.

SAN FRANCISCO, CAL.

PHYTOPATHOLOGICAL NOTES

Lolium Infected with Bunt of Wheat. Two species of Lolium were infected with bunt of wheat in artificial inoculation tests conducted in 1930-31. The seed of these species was coated with an inoculum that included both Tilletia tritici (Bjerk.) Wint. and T. levis Kühn. The inoculum contained equal parts of the 10 physiologic forms previously described by the writer. Three smutted heads of Lolium were found, 2 in L. multiflorum Lam., and 1 in L. perenne L. All 3 of these smutted heads contained spores that were typical of T. levis.

At the time the above tests were conducted the writer was quite unaware of the fact that as long ago as 1754 Tillet³ conducted similar experiments in which he obtained positive results following the dusting of the seed of *Lolium* sp. with spores of the bunt of wheat. It is not known which species of Tilletia Tillet employed in his experiments. He also obtained positive results following the inoculation of clean wheat seed with the spores of bunt obtained from Lolium.

The writer⁴ previously showed that it was possible to infect another genus, Secale cereale, with bunt from wheat. An attempt was made, therefore, to see if this organism would attack other grasses. In the fall of 1930 the following grass seeds were coated with the bunt inoculum described above: Cheat grass, Bromus secalinus; soft cheat, B. hordeaceus L.; smooth brome grass, B. inermis Leyss.; nodding wild rye, Elymus canadensis L.; English rye grass, Lolium perenne; Italian rye grass, L. multiflorum; timothy, Phleum pratense L.; meadow fescue, Festuca elatior L.; quack grass, Agropyron repens (L.) Beauv.; velvet grass, Holcus lanatus L.; proso millet, Panicum miliaceum L.; Kentucky blue grass, Poa pratensis L.; and hull-less gray oats, Avena sativa L. The cheat, Lolium, millet, and oats headed in 1931 but only the Loliums showed any infection. Removing the hulls before inoculation may enhance liability to infection.

The grasses were grown in the field in 4-ft. rows along with duplicate noninoculated check rows. No smut was found in any of the check rows. There were about 400 heads in each row of Lolium.

- ¹ Published as Technical Paper No. 179 with the approval of the Director of the Oregon Agricultural Experiment Station. Contribution from the Department of Farm Crops.
- ² Bressman, E. N. Varietal resistance, physiologic specialization, and inheritance studies in bunt of wheat. Oregon Agr. Exp. Sta. Bul. 281. 1931.
- ³ Tillet, Mathieu. Dissertation sur la cause qui corrompt et noircit les grains de ble dans les épis et sur les moyens de prévenir ces accidents. 216 pp. Bordeaux. 1755.
- ⁴ Bressman, E. N. Rye infected with bunt of wheat. Phytopath. 21: 437-440.

Bunt may appear in some of the grasses that did not head in 1931. Unfortunately, much of this sod was destroyed, so it may not be possible to determine if any of the others produce smutted heads in 1932. It is hoped, however, that some additional results may be obtained in the inoculation trials.

A microscopic examination of the spores from the infected heads of Lolium showed that they were typical of *Tilletia levis* in respect to size, shape, and smoothness of exospore. The sori, when broken, produced an exceedingly strong odor typical of wheat bunt. Figure 1 shows part of a normal and bunted head of *L. multiflorum* and a normal and bunted seed (sorus).

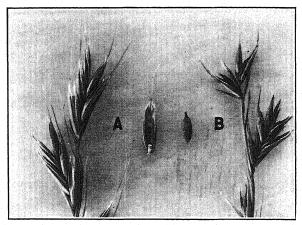


Fig. 1. Italian rye grass (Lolium multiflorum). A. Normal head and seed.
B. Bunted head and seed.

Tilletia Lolii Auersw. has been described on Lolium. It may be a form of wheat bunt, but, if so, it is more closely related to T. tritici.

Large amounts of rye grass seed are produced in western Oregon each year. There have been no reports of Tilletia here, although a species of Ustilago or Melanopsichium has been noted. It appears that Tilletia is not common. It is not known whether the T. levis reported herein consists of more than one physiologic form. If only one form is present perhaps environmental conditions at time of seeding were optimum for its germination and growth. Additional inoculation tests with the T. levis obtained from Lolium, placing it back on hulled Lolium and on wheat, are being conducted—E. N. Bressman, Oregon Agricultural Experiment Station, Corvallis, Oregon.

Eriodendron as Host of Bacterium Malvacearum.—In a commercial planting of the well-known Mexican variety "Pochote" of Eriodendron anfractuosum—the kapok tree—on the Pacific coast of Guatemala, a rather serious bacterial disease caused by Bacterium malvacearum has been found. As this organism does not seem to have been reported from any host plant outside of the genus Gossypium, the purpose of the present note will be to put this occurrence on record, at the same time adding a short description of the disease symptoms, on the new host.

The disease is most prevalent during the rainy season and more destructive on young Pochote plants grown in seed beds; after transplanting into the open field the disease takes a less destructive course. Both leaves and stems may be affected by the disease.

On young infected leaves the symptoms are more conspicuous than on the stems. On the former, irregular spots surrounded by an oily translucent halo are formed a few days after infection has taken place. As the mesophyll is attacked it dries up and becomes dark brown. If the spots are located near the points of the digitate leaves, the points generally are killed, but the damage remains insignificant. In case the spots develop near the base of the leaflets, the infected leaflet will soon drop. Young plants of about 1 foot in height have been seen to become completely defoliated in a week. As the bud scales at the base of the young leaves may also become infected the infection is readily transmitted to the developing leaf, resulting in premature defoliation.

When full-grown leaves are infected, somewhat different symptoms develop. The infection seems to take place almost exclusively on the upper leaf surface. An oily zone, typical for lesions on young leaves, is not formed. The infection spreads in this case so slowly that a protective tissue can be generated in the mesophyll, which retards the extension of the lesion. The preventive layer often succeeds in keeping part of the mesophyll and the epidermis of the underside of the leaf free from the pathogen. In this connection it must be recalled that the fully developed Eriodendron leaf is thick and leathery. Leaf petioles are also subject to infection, which invariably leads to defoliation.

When stems of young Pochote plants—1–2 months old—become infected, oblong brown lesions develop rapidly. The center of such spots turns grayish when older, and the border zone often appears slightly raised over the surface because of extensive formation of wound cork. Often such lesions remain superficial and accordingly cause only slight damage to the stem. If the growth of the stem is rapid, as it is during prolonged wet weather, lesions on the stem near the growing point may extend into the xylem. A girdle of necrotic tissue forms around the main axis, thereby causing the death of the growing point. This leads in its turn to the formation of one or several new shoots below the girdle. As this procedure may be repeated

several times during the growing season, the whole habitus of the Eriodendron tree may become completely changed. A healthy young tree normally attains a height of about 5 feet before the first whirl of branches is formed. In case of repeated girdling of successive bud shoots, a stunted, irregularly branched dwarf tree results. Such trees are, of course, useless for planting purposes.

Similar bacterial lesions have also been found near the growing point on the horizontal branches of older trees. The result is the same as just described, with the difference, however, that the new shoots, instead of growing horizontally, like the mother branch, grow upwards at a right angle to the supporting branch. This negative geotropism of adventitious Eriodendron shoots is always displayed when horizontal branches have been seriously wounded and have given rise to new shoots.

A series of cross-inoculation experiments proved that Bacillus malvacearum from cotton readily infected the Eriodendron, causing lesions of the type described above in 6 to 10 days. Infections with the bacterium from Eriodendron similarly caused infection on Gossypium.—B. T. Palm, Department of Botany, University of Illinois, Urbana, Illinois.

A Note on Entyloma Dahliae Syd. from Sumatra and Guatemala.—A disease of the cultivated Dahlia, caused by Entyloma Dahliae Syd., has recently been causing some alarm to horticulturists on the Continent of Europe. Originally described from South Africa, the Entyloma has subsequently, some years ago, been found also in Spain and in Holland. At present the fungus would seem to be spreading quite rapidly over the European Continent, having been reported of late at least from Belgium, France, and Germany by pathologists in the respective countries.

To the above list of countries can now be added Sumatra (Dutch East Indies) where the Dahlias, which are cultivated there only at higher altitudes, were found rather badly affected by this Entyloma disease in a number of mountain localities; these observations were made in 1924 and 1925. In all cases the plant material had been introduced from Holland by several importers, indicating that the disease must be fairly wide-spread in the last-mentioned country.

As Entyloma Dahliae seems to belong to the growing number of pathogens that, upon introduction, show a rapid extension over the Continent of Europe, the following tentative suggestion regarding its country of origin may be of interest.

The Dahlias are, as is well known, natives of Mexico and Guatemala, where they occur at altitudes of 6,000 ft. and higher above sea-level. During a stay in Guatemala the writer had the opportunity of studying two species, *Dahlia excelsa* Benth. and *D. coccinea* Cav., both of which are quite common on the volcanoes near Antigua. It was found that a species of

Entyloma, which to all purposes agreed very well with the descriptions of E. Dahliae Syd., occurred, often in great abundance, on D. coccinea, not, however, on D. excelsa. The same fungus was found prevalent also on cultivated Dahlias in gardens around the city of Guatemala. After the first indication of this limited host range had been obtained, during several growing seasons (1927–29) a search was made for the Entyloma on D. excelsa, always, however, with the same negative result. This fact probably is not without significance, as D. coccinea is regarded as one of the ancestors of our cultivated Dahlia, whereas D. excelsa would seem to have been introduced into cultivation but recently in Mediterranean countries. Recent importations of the former species to botanical gardens or by horticultural establishments for crossing purposes may thus possibly account for the introduction and subsequent distribution of Entyloma Dahliae on cultivated forms of Dahlia.—B. T. Palm, Department of Botany, University of Illinois, Urbana, Illinois.

An Undescribed Loose Smut of Barley. As a result of further studies on the infection of barley by the loose-smut fungus through seed inoculation discovered by Tisdale and Tapke, it has been found that loose smut of barley is caused by either of two fungi: (1) Ustilago nuda (Jens.) Kell. and Sw. and (2) a darker-spored species for which the name Ustilago nigra n. sp. is proposed. The two smuts are readily separable, as shown below.

Bases of comparison	Ustilago nuda	Ustilago nigra
Color of spore mass	Olivaceous brown Golden brown 5.5 × 6 µ 3 to 6 months, rarely 1 year	Dark chocolate brown Dusky brown $6.5 \times 7 \mu$ Over 18 months
Control of loose smut in plants from seed from inoculated flowers fol- lowing treatment of the seed with Ceresan dust or liquid formalde- hyde (1-320, seed soaked 90 min- utes)	No control	Complete control
Ability of the fungus to cause seed- ling infection resulting from inocu- lation of mature seed with chlamy- dospores	No seedling infection	Seedling infection

a Limited studies.

¹ Tisdale, W. H., and V. F. Tapke. Infection of barley by *Ustilago nuda* through seed inoculation. Jour. Agr. Res. 29: 263-284. 1924.

In the light of the above, the divergent results obtained by investigators in the production of loose smut of barley through dusting mature seed with chlamydospores and in the control of loose smut through seed treatment with surface disinfectants, might be explained.—V. F. TAPKE, Bureau of Plant Industry, U. S. Department of Agriculture, Washington, D. C.

Plant Pathology at the Fourth Congress of the International Society of Sugar Cane Technologists in Puerto Rico.—The 4th Congress of the International Society of Sugar Cane Technologists was held in Puerto Rico, Mar. 2 to 16, 1932. The section on diseases of sugar cane was attended by G. Wilbrink, of Java; A. F. Bell, of Australia; Atherton Lee, of Philippine Islands; J. P. Martin, of Hawaii; E. W. Brandes and E. V. Abbott, of the United States, and Melville T. Cook, C. E. Chardon, R. A. Toro, Arturo Roque, and J. G. Harrer, of Puerto Rico.

Three sessions were devoted entirely to diseases of sugar cane and a joint session was held with the entomologists.

Thirty papers were presented, including an international survey of the diseases of sugar cane, among which were 7 papers on virous diseases; 7 on bacterial diseases; 2 on root diseases; and 12 papers on other subjects. A 5-day excursion through the sugar-cane districts of the Island gave an excellent opportunity for the study of the diseases in the field and to visit the 2 experiment stations. The papers will be published in the proceedings of the society. The next Congress will be held in Australia in 1935.—Mel. T. Cook, Insular Experiment Station, Rio Piedras, Puerto Rico.

Citrus Mildew.—In Phytopathology 9: 266, a correction is made on citrus mildew by Mr. T. Petch to a note over his name in Phytopathology 5: 350-352, in which he indicates that this fungus, which closely agrees with Oidium tingitaninum Carter, by which name it is now usually referred to in Ceylon literature, does not occur in Ceylon on pummelo (Citrus maxima Merr. = C. decumana L.).

A recent revision of the various Citrus species on which this mildew has been observed makes a further correction desirable. Citrus mildew is now commonly found in Ceylon on pummelo (Citrus maxima Merr.), grapefruit (C. maxima Merr. var. uvacarpa Merr. & Lee), as well as on sweet orange (C. sinensis Osbeck), lemon (C. limonum Risso), and Mandarin orange (Citrus species undetermined) as previously recorded. In addition, it has been found also on the Kalamondin orange (C. mitis Blanco) and, more commonly, on the Ceylon sour orange (Citrus species undetermined). No perithecial stage of this Oidium has been observed up to date. It has been found that this mildew can be very satisfactorily controlled by regular weekly sprayings during damp warm weather with certain lime-sulphur mixtures.—W. C. Lester-Smith, Department of Agriculture, Peradeniya, Ceylon.

BOOK REVIEW

Waksman, Selman A. Principles of Soil Microbiology. XXVIII+894 pp., XV pls., 83 figs., 2nd Ed. Thoroughly Revised. Williams and Wilkins Company, Baltimore, Md. 1932. Price \$10.00.

Research workers and teachers in soil microbiology are well acquainted with the first edition of this book. For the information of others, it may be said that Waksman brought together the available information on soil microbiology, the first time that such a review of literature on the subject has appeared in English. The works of the Russian and other foreign investigators whose publications are generally inaccessible to the average worker were cited and their results summarized in the discussion of the various subjects.

In the preface to the second edition, the author states, "Within the brief period of four years, since the appearance of the first edition of this book, the numerous contributions to our knowledge of the rapidly growing subject of microorganisms and their activities in the soil necessitate a number of changes in the new edition. The author availed himself of the criticisms which have been so freely given in the various reviews of the first edition".

It is only natural that the second edition of a work of this kind would have many changes. New material is constantly becoming available, which, of course, means another edition in a few years. The typographical errors and the mistakes in citations in the first edition have been largely corrected, although some persist, such as spelling Shantz as Shanz throughout the book. In such a large undertaking, minor mistakes perhaps should be overlooked (author index—E. J. Russell XV should be E. J. Russell XIV; F. Löhnis XVI should be F. Löhnis XV, may serve as illustrations).

The author has adhered to the original plan of the work, dividing it into four major parts.

Part A. The soil population. Occurrence and abundance of microorganisms in the soil. Two chapters.

Part B. Isolation, identification, and cultivation of soil microorganisms. Twelve chapters.

Part C. Chemical activities of microorganisms. Seven chapters.

Part D. Soil microbiological processes and soil fertility. Thirteen chapters.

In comparison with the first edition, Part A has one more chapter; Part B, one less; Part C, two less; and Part D, four more.

In order not to increase the size of the book but still include the literature of the past four years, it was necessary to omit something. Waksman

chose to omit the titles of the original papers, to combine certain chapters, and to omit others. Several new chapters, however, have been added, so that actually the saving of space is due mainly to the omission of the titles of the references cited. A great many will undoubtedly criticize the author for doing this instead of finding some other way of limiting the size of the volume. However, to many this will be of minor importance, since the work is indexed according to author and subject.

The criticism might be offered that a number of citations are often placed under one reference number at the bottom of the page without any distinguishing marks except that the volume numbers are in black-face type. From the view-point of the reader, this is unfortunate, especially, since some of these citations may be referred to on subsequent pages. The summaries at the end of certain chapters appear to add nothing of value and might be omitted.

The new edition contains about a thousand more references than the first and a few more figures and tables. There are four less plates. Plate I has new illustrations and plates V and VI have been changed and improved.

The task of collecting and summarizing the results of researches on soil microbiology can be appreciated only by those who attempt to keep abreast of the science. Therefore, the value of the book under discussion need not be proclaimed; it speaks for itself.—Nathan R. Smith, Soil Microbiology, U. S. Department of Agriculture, Washington, D. C.

ABSTRACTS OF PAPERS DUE NOVEMBER 15

November 15 has been designated as the closing date for receipt of manuscript for phytopathological abstracts. This means that abstracts must reach the Secretary not later than November 15. They must not exceed 200 words in length and three typewritten copies are necessary. Indicate time required for presentation and whether stereopticon will be needed.

In editing these the committee on abstracts will apply the following rules:

- Not more than two abstracts under sole or senior authorship of one person will be accepted.
- 2. Previously published material must not be included.
- 3. Abstracts must not exceed 200 words.





LEIGH HUMBOLDT PENNINGTON

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LEIGH HUMBOLDT PENNINGTON 1877–1929

PERLEY SPAULDING

Doctor Leigh H. Pennington died suddenly from heart disease on April 24, 1929, while in Washington, D. C., carrying on investigations of the white-pine-blister rust for the Federal Bureau of Plant Industry during sabbatical leave. He had worked the day before and until 10 o'clock at night at his office, apparently in normal health. When he did not appear at the usual time in the morning investigation revealed his death. He was 51 years of age. He leaves his wife and one daughter, Mrs. Phyllis Percival.

Doctor Pennington was a son of Claribel (Pratt) and Byron Humboldt Pennington. He was born October 26, 1877, in the crossroads hamlet of Pennington's Corners in the town of Macon, Michigan. The family lived in the original home of his grandfather. The grandfather, Israel Pennington, moved about 1820 from Farmington, near Canandaigua, Ontario County, New York, to Pennington's Corners. The hamlet evidently took its name from this family, members of which even yet occupy the land in that locality, although it is owned by Henry Ford, who has preserved an old-time community in all of the original simplicity that is now possible. quite a large nursery was established and maintained by the grandfather. At the death of its original owner the father continued the business and a large part of it still stands on the original location. The grandfather was a great student, reciting poetry while at work in the field and making a real study of his work. The mother taught in a seminary until her marriage. The father was a civil engineer, graduating at the University of Michigan. The family was thus well above the average in education and was in a leading position in its community. The boy very naturally studied plants and adopted teaching as a livelihood. Experience about the nursery and more or less conscious imitation of his father undoubtedly trained him in the habit of carefully noting details. His memory was excellent, so that he depended upon it more than most people can. In the end, this was unfortunate, as it resulted in much of his last work being lost because of lack of records of things perfectly well known to him but unknown to any one else.

His early training was in the public schools of Macon and high school of Tecumseh, Michigan. Later he taught in the public schools of Macon

and near-by towns for 3 years. He was principal in the Palmyra High School for 5 years. At this time he met his future wife, Miss Blanche Van Fleet. They were married in 1902. In 1904 he entered the University of Michigan and received the degree of A. B. 3 years later. He at once began post-graduate work with Doctor Newcombe at the same university, being given the degree of Doctor of Philosophy in Botany in 1909. During the last 4 years he was assistant in the Department of Botany. One year was spent as instructor in botany at Northwestern University, after which he went to Syracuse University as Assistant Professor of Botany in the College of Liberal Arts. Two years later he became Associate Professor. In 1914 a Department of Forest Botany was established in the New York State College of Forestry of Syracuse University and he was made Head Professor, which post he held until his death.

During the summer vacations of the college Doctor Pennington occupied his time with investigations of forest-pathological problems. He served as expert in the United States Department of Agriculture in the summers of 1911 and 1912; collaborator of the New York Botanical Garden in 1913 and 1914; since 1917 as pathologist or collaborator with the United States Department of Agriculture, engaged in studies of the white-pine-blister rust, specializing in its epiphytology. These studies continued until his death. One entire sabbatical year was thus spent in British Columbia.

Doctor Pennington was a fellow of the American Association for the Advancement of Science, member of the Botanical Society of America, The American Phytopathological Society, the American Ornithologists Union, the American Genetics Association, the Society of American Foresters, the New York State Forestry Association, and the fraternities Sigma Xi, Phi Kappa Phi, and Alpha Xi Sigma. He was a 32nd degree Mason. He was active in the University Methodist Church and was interested in many philanthropies. He always tithed his income. He was constantly helping students out of their difficulties, being exceedingly liberal of his time, interest, and money.

Doctor Pennington was most devoted to his family, a progressive member of his community, and a loyal and devout member of his church. He was characterized, above all, by his sterling honesty and common sense. Whatever the situation, he instinctively chose the sound plan of action. Clear thinking naturally resulted in the logical and best action. Like many strong thinkers, he was very unassuming. When an opinion was once formed it was held tenaciously unless his opponent could convince him that he had formed it on false premises. He was a staunch friend when one once really penetrated his somewhat austere exterior. He was a successful teacher, beloved by his students to an unusual degree. He was always ready to

help his boys and had their confidence and good-will. Had he chosen to push himself to the front, he undoubtedly could have been successful in a much more prominent way, but that would have been distasteful to him in the highest degree. In all his various activities he chose some main objective and strove for it, ignoring trifles and distractions. His counsel was sought and heeded both by students and in matters of policy of the college.

In his profession he was an intensely hard worker, although his unhurried manner would often belie that fact. He was a keen observer of human nature, a thorough student, and an excellent scientific investigator. He had a keen sense of humor that was shown on occasion by quiet chuckles rather than gales of laughter. He was meticulously careful of details, possibly due to an incident of his early youth. On learning that his boy was collecting birds' eggs, his father required him to record properly all the collection data for each set taken so that the collection would have real scientific value. He was strong in initiative and sometimes persisted in a line of action almost to the point of foolhardiness, it seemed to his friends. But, with all this, he was resourceful and self-reliant, so he probably did not overstep the limits of his abilities.

His absolute sincerity gave him an attractive personality to those who look beneath the surface. His reserved manner, resulting from his strong self-control and equanimity, sometimes threw people off the track when they first met him. His pronounced initiative made him an excellent leader on expeditions of all sorts. The writer has accompanied him on trips of different lengths and difficulty; has, with him, listened to the courting song of an approaching black bear in the Adirondacks at dusk; inspected newly made bear tracks, not yet filled with muddy water, in grizzly-bear country in British Columbia; and the same self-possession and poise were always evident. The writer's first airplane trip was made with him as companion in the forward cockpit of a 4-man hydroplane on the coast of British Columbia. Hardly had the machine taken off in a heavy head wind before the engine died and a forced landing had to be made in the main channel with waves running several feet high. The force of the landing broke the slatted seat under him, but he turned not a hair. He suffered from terrific headaches occasionally and undoubtedly knew that a form of indigestion was the cause. He made considerable study of suitable foods and was a good camp cook for a small party. He knew the uses of native plants, especially mushrooms, and often eked out a limited larder on trips by using them.

Professionally, Doctor Pennington was considered one of the soundest workers in forest pathology. He prided himself on being the only full professor in forest pathology in the country. He was a good mycologist. In this as in other lines of work he realized that specialization was the order of the day and made himself the authority on the genus Marasmius, publishing on this genus for temperate North America in "North American Flora." In the same methodical and thorough manner he worked several years on the white-pine-blister rust with respect to the time of its introduction into various regions of this continent, its rate of spread from various centers, its means of spreading, and its probable final range. Indeed, he was working on this problem at the time of his death. In accounting for its extensive and rapid spread in British Columbia his knowledge of the birds came into play, so that he was able to eliminate them as carriers of the disease over long distances.

He collected birds' eggs from boyhood with scientific care and accuracy. Exchanges and purchases made his one of the notable private collections in the country. He specialized on the Raptores and the birds of New York State. He also had a valuable library on ornithology. He knew the birds well for an amateur unable to devote much time to his hobby. While in British Columbia, studying the spread and past history of the white-pine-blister rust there, he made himself familiar with the birds, being there an entire year, so that he saw the migrating as well as the resident species. It was the writer's good fortune to see the spring migration of 1922 with him. With his assistance notes were made on the species observed, which were considered valuable by the U. S. Bureau of Biological Survey, as they were made in a region where such observations were lacking in their records.

He was also deeply interested in theology and New Testament translations. He was a keen collector of such books. Just before his death he was studying Greek so that he could read the New Testament in that language.

Such a man cannot be replaced in his profession, community, or church. Such a character is too rare in this imperfect world to be duplicated in the same community.

The circle is broken,—one seat is forsaken,—
One bud from the tree of our friendship is shaken,—
Wordsworth.

Division of Forest Pathology, New Haven, Conn.

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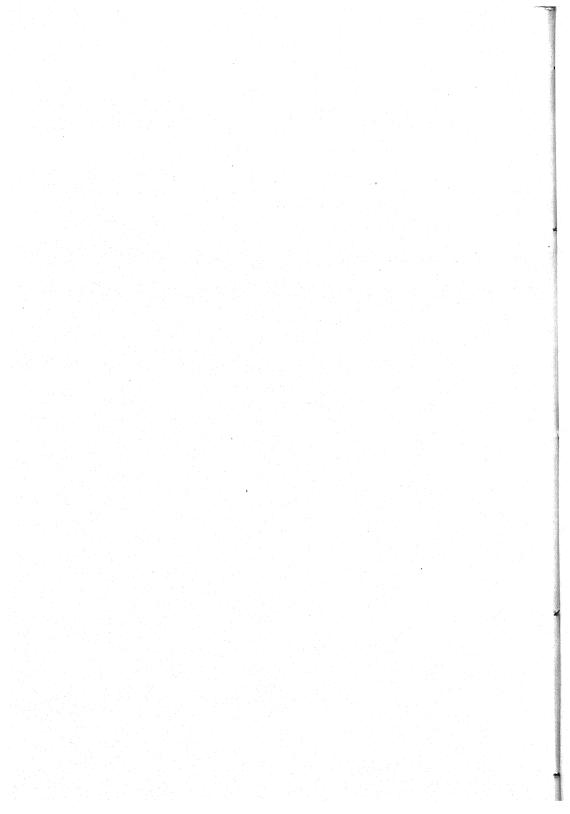
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SOME PROBLEMS CONCERNING BLUE MOLD IN RELATION TO CLEANING AND PACKING OF APPLES¹

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INTRODUCTION

During storage of the Washington apple crop each season much loss is sustained from diseases. Exact figures indicating the amount of this loss due to specific organisms are difficult to obtain. Pailthorp and Park (9) reported that an average of 21.3 per cent of the rejections of Washington car shipments in the crops of 1922-1925 on eastern terminals were due to decay. It is generally conceded that blue mold is the cause of the greater part of this loss. Brooks, Cooley, and Fisher (1) report that blue mold causes from 80 to 95 per cent of the total rots of the crop. Heald et al. (4) reported an average of 3.76 per cent blue-mold decay in 2,102 cars of the 1925 crop and 3.65 per cent in 1,795 cars in the 1926 crop on eastern terminals. Heald and Ruehle (5) found that condition reports of the 1929 Washington apple crop at the time of movement from local storage showed many cars with 2 to 3 per cent decay, a considerable number 3 to 7 per cent, and a smaller number 8 to 10 per cent, while some few lots showed from 12 to 40 per cent blue mold. They stated that at least 75 per cent of the storage rots in apples from the principal Washington districts are due to Thom (12) and Heald and Ruehle (5) reported that Penicillium expansum Link is the most important blue-mold species in the cause of apple decay.

The passage of the Federal Food and Drug Inspection Act limited the arsenical load of apples for domestic sale in 1927 to 0.025 grain arsenous oxide per pound of apple, and this figure was reduced to 0.012 grain by 1931. Since the Act went into effect the extensive investigations on fruit-cleaning methods have shown that washing the fruit with various solvents is superior to other methods. As now employed these solutions contain either hydrochloric acid or some of the various alkaline compounds.

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Hydrochloric acid is more commonly employed because of its cheapness, ease of use, and slight danger of injury to fruit. However, in the 1931 season a number of packing plants changed to alkaline washes in order to reduce the arsenical deposits to the tolerance. These solutions have been commonly used in the 1930 and 1931 seasons at temperatures up to 110° F. and sometimes 120° F. in order to facilitate cleaning. It is the experience of growers and investigators (Diehl et al., 2; Heald and Ruehle, 5) that temperatures of 120° F. or more tend to bleach fruit and may lower the keeping quality.

As early as 1907 Smith (10) found that the washing tanks were one of the main sources of infection of brown rot of lemons and tested various disinfectants to reduce the spore load of the tanks. It was later found that other decays of citrus fruit were spread in a similar manner. Various compounds have been employed in the citrus industry in an attempt to counteract the effect of this inoculum bath and decrease the amount of decay. For a time borax was used, but, since 1927, sodium bicarbonate has been employed.

Heald et al. (4) found that the apple-washing tank was one of the main sources of contamination of apples and that an increase of the spore load increased the percentage of decay. Treating the tanks during the night with a formaldehyde solution to keep the spore load reduced from day to day was recommended. This has since been recommended by other workers but has not been generally adopted.

No practical treating solution for the control of blue mold of apples in a manner comparable to that in use in the citrus industry for blue and green molds has yet been found. The similarity of the two washing treatments and the fact that it does reduce decay in citrus fruits have led firms selling various washing compounds to claim that these substances greatly reduce blue mold of apples and that they are toxic to *Penicillium expansum* spores. Fisher and Reeves (3) reported that the washing solutions have little or no toxicity to fungous spores, and Diehl *et al.* (2) reported that spores of *Gloeosporium perennans* Zeller and Childs were not killed by 6 days' exposure to either acid or alkaline washing solutions.

The extreme resistance of the spores of Penicillium species to various agents has long been known. Various species have been reported growing on strong copper sulphate solutions and concentrated sugar solutions. Of the fungi studied, Stevens (11) found that *P. crustaceum* Fries was the most resistant to acids, alkalies, and salts.

The purpose of this investigation was to determine the degree of toxicity of 3 of the most commonly used washing solutions to spores of *Penicillium expansum* and to investigate some of the factors that would be involved in

the use of a treating solution for lessening decay. A practical method of reducing the spore load of some of the sources of contamination of the washing tank was examined.

MATERIALS AND METHODS

In this investigation the cultures used were transfers from the Penicillium⁴ found by Heald and Ruehle (5) to be most abundant in occurrence and to cause the most rapid decay of apples. Unless otherwise mentioned the spores used in this work were taken from 5- to 9-day-old, vigorously growing plates of *P. expansum*, and no old dried cultures or young plates just beginning to fruit were employed.

Spore suspensions were made by placing spores scraped from the surface of the Petri-dish cultures of the organism into a flask containing 300 cc. sterile distilled water and shaking vigorously for 5 minutes to break up the spore clumps. Five cc. of this suspension were transferred with a sterile pipette to flasks containing 300 cc. sterile distilled water plus the desired quantity of the compound whose toxicity was being tested, and shaken for 5 minutes. Before each flask was to be tested for spore viability it was thoroughly shaken to insure uniformity of spore suspension. Unless otherwise stated 1 cc. of this suspension was transferred with a sterile pipette to each of three 9-cc. sterile water blanks, and these were simultaneously shaken. One cc. of each of these 1 to 10 dilutions was transferred with a sterile pipette to sterile Petri dishes. Two per cent potato dextrose agar cooled to about 42° F. was poured over this transfer and the dish rotated. In the early series the cheek test of estimation of the temperature was employed. In later series, in order to eliminate the temperature variable, the agar was melted and allowed to cool nearly to the solidifying point. It was then placed in an incubator maintained at a temperature of 42° F. for from 5 to 12 hours in order to standardize the temperature. No more fluctuation occurred in tests on media of estimated temperature than on those of known temperature. Plates were incubated at room temperature. When the colonies first began to show green coloration counts were made by inverting the plates over a Spencer substage lamp. The entire plate was counted in sections outlined with wax pencil. Thus the figures in the tables in this paper are averages of the 3 plates made from each flask at each viability test and are expressions of the approximate number of viable spores per cubic centimeter in the solution at the time the test was taken.

During the 1930 and 1931 seasons packing plants used hydrochloric acid at a strength of 1 to 3 per cent by volume and kept it at the desired acidity by titration. In this study commercial grade HCl was used at 3 per cent

⁴ Identified by Dr. Charles Thom as Penicillium expansum Link.

by volume, or, in terms of the experiment, 9 cc. of concentrated commercial HCl to a 300 cc. water blank. Measured by the hydrogen-electrode method, this gave a solution of pH 1.2.

The commercial cleaner of the sodium carbonate-borax type⁵ was reported by the company to consist of 100 parts of soda ash to 1 part of borax in the 1930 season. It was used at the rate of $\frac{1}{2}$ – $1\frac{1}{2}$ lb. per gallon of water. Technical caustic soda flakes were added in cases where the fruit was difficult to clean, and then never over $\frac{1}{2}$ oz. per gallon of solution. The ingredients are shipped separately to the packing plant and mixed as used. The cleaner was used in this study at the rate of $1\frac{1}{2}$ lb. per gallon (without caustic soda, as this substance was rarely used in washing operations). Materials used were the soda ash and borax supplied by the company from their commercial stock. In terms of the study the strength was 53.91 gm. per 300 cc. water blank, giving a solution with a pH value of 10.3 measured by the hydrogen electrode.

The commercial cleaner of the sodium carbonate-trisodium phosphate type⁶ was reported as composed of equal parts of each constituent and used at the rate of 60 lb. per 100 gal. of water in the 1930 season. In 1931 it was used in varying proportions at greater strengths, one case of 300 lb. per 250 gal. being known. The ingredients of this cleaner are mixed at the factory. Material used in this study was the stock mixed powder supplied by the company. In terms of the experiment the strength was 21.5 gm. per 300 cc. water blank. The solution measured pH 10.3 by the hydrogenelectrode method.

In order to obtain the effect of the solutions at different temperatures the flasks were placed in an incubator, the temperature of which did not fluctuate more than 2.5° F. in 1 week. The flasks were placed in pans of water to stabilize the temperature and to reduce the evaporation of the solutions that would have increased the concentration of the treating compound.

The temperature of the flasks was stabilized by keeping them in an incubator for 10 to 12 hours at the desired degree, after which the spores were added. Transfers from the flasks were made in a sterile culture chamber and usually required not more than 10 minutes. The temperature fluctuation of the solutions was slight during that time.

Time required for germination, percentage of germination, amount of growth of the germ tube in a given time, and other criteria have been used as measures of toxicity of substances by other workers. As the spores are in suspension in the solutions in the washing tanks and are directly sub-

⁵ Brogdite cleaner, supplied by the Northwest Brogdex Co., Yakima, Washington.

⁶ Laux cleaner, supplied by The Laux Laboratories, Seattle, Washington.

jected to both chemical toxicity and temperature factors, it was deemed advisable to duplicate as nearly as possible these conditions in the tests. A delaying of germination due to one or both factors involved is of no great importance in the ultimate fruit decay because of prolonged storage periods. The criterion of spore viability adopted was the ability to form colonies on 2 per cent dextrose potato-agar.

The degree of experimental variation with this technique was determined (Table I). Two flasks of 300 cc. sterile distilled water were inoculated

TABLE 1.—Number of viable spores of blue mold per cubic centimeter obtained in replicated plates from the same spore suspension

751	771 7 7	Fla	ısk 2
Plate	Flask 1	A Series	B Series
1	3,430	2,250	
2	3,700	2,460	
3	3,700	2,330	
4	4,710	2,150	
5	4,650	2,160	
6	3,660	2,200	
7	3,570	2,470	
8	3,500	2,180	
9	3,330	2,260	2,250
10	3,140	2,750	2,540
11	3,250	2,100	2,140
. 12	3,990	2,010	2,240
13	3,320	2,430	2,130
14	3,200	2,160	2,120
15	3,550	2,100	2,240
16	3,130	2,220	2,110
17	3,210	2,340	2,180
18	2,470	2,080	2,170
19	4,270	2,330	2,120
20	2,990	2,180	2,060
Average of series	3,523	2,258	2,191

with a spore suspension and 20 plates made at once from each. The A and B series of flask 2 show the possible variations of 2 plates made from the same dilution tube with the same pipette.

When high spore concentrations were employed the range of experimental variation was fairly broad, and, hence, only large constant changes in number of viable spores per cubic centimeter are significant in the experiment. Within limits, however, there is considerable uniformity of results.

EFFECTS OF SPORE AGE ON RESISTANCE TO HEAT AND TOXIC COMPOUNDS

The factor of spore age has been shown to have some effect on resistance to both temperature and toxic substances. Henderson Smith (6) found that the resistance of Botrytis spores to phenol increased with the age, and Marloth (8), using alkaline solutions, states, "The resistance of 60-day-old spores against the treating substances as compared with 14-day-old is quite marked for *P. digitatum*." The effect of spore age on resistance to the temperature factor in these experiments is shown in table 2. Two flasks of 300 cc. sterile distilled water were inoculated with 5 cc. of a suspension of spores from 5-day-old and 49-day-old cultures, respectively. These were incubated at 110° F. and plates made at stated intervals.

TABLE 2.—Number of viable spores of blue mold per cubic centimeter obtained at intervals from water suspensions of young and old spores incubated at 1.10° F.

Time		Flask with 49-day- old spores	Flask with 5-day- old spores
0 hours		2,376	2,426
1 hour lat	er	1,200	990
2 hours '		1,426	1,020
3 " "		1,600	1,170
4 " "	4	1,490	1,460
5 "	() · · · · · · · · · · · · · · · · · ·	1,050	1,286
6 " "	'	903	1,180
7 11 1		826	980
8 "	4	743	896
10 "		133	106
12 "		106	2
			<u> </u>

It appears that there is no significant difference in resistance to temperature as a result of differing age.

The effect of spore age on resistance to the treating solutions is shown in table 3. Two 300 cc. sterile distilled water blanks containing 3 per cent by volume of commercial hydrochloric acid were inoculated with suspensions of spores from 5-day-old and 54-day-old cultures. Petri-dish transfers from these flasks were made at indicated intervals. The results indicated that older spores were considerably more resistant to hydrochloric acid than those more recently formed, paralleling the results of Marloth (8) with alkaline solutions and Henderson Smith (6) with phenol.

The resistance of 9-day-old and 44-day-old spores to a hypochlorite solution was determined (Table 4). Sodium hypochlorite solutions were found, in general, to have a high toxicity to *Penicillium expansum* spores. A

TABLE 3.—Number of viable spores of blue mold per cubic centimeter obtained at intervals from suspensions of old and young spores in acid cleaner at room temperature

Time	54-day-old spores	5-day-old spores	
0 hours	2,916	5,180	
41 '' later	3,363	4,020	
91 (6 (6	3,470	2,770	
18½ " "	3,190	1,703	
26 '' ''	3,033	990	
343 " "	2,616	396	
48 '' ''	2,330	76	
70½ " "	973	0	
96 '' ''	33	0	
126 " "		0 9 9 9	

commercial solution of this type⁷ (testing 5.7 per cent available chlorine by the acetic acid method against a standard sodium thiosulphate solution) was used at the rate of 1 cc. to a 300 cc. sterile distilled water blank. Triplicate flasks held at room temperature were used for each series and plates made in triplicate from each at indicated intervals.

TABLE 4.—Number of viable spores of blue mold per cubic centimeter at intervals in water suspension of young and old spores plus hypochlorite solution

m:	9	-day-old spo	res	44-day-old spores		
Time	Flask 1	Flask 2	Flask 3	Flask 1	Flask 2	Flask 3
0 min,	2,660	2,680	2,700	2,360	2,480	2,750
21 11	1,960	2,190	2,790	2,280	2,410	2,680
5	2,990	2,510	2,630	2,350	2,460	2,530
7½ "	2,830	2,650	2,640	2,400	2,380	2,490
10 ''	1,450	2,670	2,710	2,330	2,310	2,660
121 "	80	320	530	2,210	2,050	2,700
15 ''	80	230	440	940	1,660	2,640
17½ "	60	110	40	280	290	750
20 '' 🕶	50	120	150	100	110	400

The resistance of blue-mold spores to the toxic substances tested increased with the aging of the spores, probably because of lowered water content, changes in the protoplasm and cell wall and retarded life processes.

Five-day-old and 9-day-old spores were placed in 300 cc. water blanks plus 3 per cent commercial hydrochloric acid and held at room temperature. Variation in the lethal action due to the 4-day difference in age did not

⁷ Clorox, manufactured by Clorox Chemical Co., Oakland, California.

exceed the normal experimental error. Any group of spores obtainable would also be of varying ages due to the manner of their development on the conidiophores.

THE RELATION OF PH OF MEDIUM TO THE GROWTH OF COLONIES

The hydrogen-ion concentration of dilution water blanks and of poured plates made from them was determined by the colorimetric method (Table 5).

TABLE 5.—The pH of 1-10 dilution tubes and corresponding Petri-dish cultures when cleaning mixtures are added

	pH concentration			
Solution	1-10 dilution tube	Petri dish		
Sodium carbonate-borax cleaner, 1½ lb. per gal. Sodium carbonate-trisodium phosphate	above 9.6	8.2		
cleaner, standard solution	above 9.6	6.6		
tion	below 1.8	5.3		

As the pH of ordinary 2 per cent dextrose potato-agar is about 6.2 these fluctuations from the treating solutions might have contributed to variations in the results. As no information on the relation of hydrogen-ion concentration to growth of Penicillium expansum was found in the literature, this factor was considered. The shortcomings of the system of growing an organism on plate cultures to determine its pH relations (buffer action of the medium and the autolytic action of the organism), as pointed out by Marloth (8), are realized. It seemed desirable to use the following method. however, as the organism was being grown in plates in the experiment. Two per cent dextrose potato-agar was, accordingly, titrated by the colorimetric method, when either a dilute HCl or NaOH solution was added to obtain the different H-ion concentrations. A single, nonsporulating, minute colony of P. expansum developed from spores thinly scattered on 2 per cent dextrose potato-agar plates was planted in the center of each Petri dish. The plates were incubated at room temperature. At pH 2.3 the medium was completely liquefied, so that normal growth did not occur. The measurements in centimeters across the greatest diameters of the colonies at the end of 7 days are given in table 6. Apparently the growth of the organism was not greatly affected by the hydrogen-ion concentration within the ranges tested. For this reason little fluctuation in growth occurred from the changes in pH of the medium from the addition of treating solutions to the spore suspension.

TABLE 6.—The diameter in centimeters of colonies of blue mold after 7 days on media of different pH values

pH 2.3	3.8	4.6	5.5	6.2	7.0	8.0	9.6
1.5	5.3	5.2	5.3	5.0	4.9	4.8	4.7
1.6	5.4	5.2	5.1	5.1	4.9	4.4	4.8
1.6	5.0	5.0	5.2	5.1	4.9		4.9
1.5	4.8	5.3	5.3	5.1	5.2		4.8
1.55	5.12	5.17	5.25	5.07	4.75	4.6	4.8
	1.5 1.6 1.6 1.5	1.5 5.3 1.6 5.4 1.6 5.0 1.5 4.8	1.5 5.3 5.2 1.6 5.4 5.2 1.6 5.0 5.0 1.5 4.8 5.3	1.5 5.3 5.2 5.3 1.6 5.4 5.2 5.1 1.6 5.0 5.0 5.2 1.5 4.8 5.3 5.3	1.5 5.3 5.2 5.3 5.0 1.6 5.4 5.2 5.1 5.1 1.6 5.0 5.0 5.2 5.1 1.5 4.8 5.3 5.3 5.1	1.5 5.3 5.2 5.3 5.0 4.9 1.6 5.4 5.2 5.1 5.1 4.9 1.6 5.0 5.0 5.2 5.1 4.9 1.5 4.8 5.3 5.3 5.1 5.2	1.5 5.3 5.2 5.3 5.0 4.9 4.8 1.6 5.4 5.2 5.1 5.1 4.9 4.4 1.6 5.0 5.0 5.2 5.1 4.9 1.5 4.8 5.3 5.3 5.1 5.2

THE RELATION OF TEMPERATURE TO SPORE GERMINATION

In order to determine the effect of different temperatures on spore germination, tests were conducted with sterile distilled water, tap water, and 2 per cent apple juice in duplicate. The pH values of these solutions were 7.9, 8.6, and 4.3, respectively. No germination had occurred at the end of 2 weeks in the tap water and distilled water incubated at room temperature and at 90° F. The spores germinated readily when plated out with 2 per cent dextrose potato-agar. When 2 per cent apple juice was used no germination occurred in 14 days at 90° F.; but, at room temperature, there was no germination in 4 hours, 15 per cent in 12 hours, 20 per cent in $27\frac{1}{2}$ hours, 25 per cent in $37\frac{1}{2}$ hours, 35 per cent in $47\frac{1}{2}$ hours, 40 per cent in 76 hours, 40 per cent in $126\frac{1}{2}$ hours, and 40 per cent in 14 days. This low germination was due to the extreme spore concentrations used. Spores from these flasks germinated readily when plated out with 2 per cent dextrose potato-agar at the end of 14 days.

Examination of the spores in the flasks of the washing solution showed in all cases that they had not germinated. Any toxic action exerted by the solutions tested is, therefore, on the spores themselves and not on the germ tubes.

The germination of *Penicillium expansum* spores at 32° F. was determined. Eight-day-old spores were placed in 50-cc. flasks containing 25, 50, and 100 per cent, respectively, of sterile apple juice. One flask of each was incubated at room temperature, and 2 were held in an electric refrigerator at 32° F. In 12 hours some germination was noted in the 25 and 50 per cent solutions held at room temperature. In 23, 48, and 72 hours germination was evident in all the flasks held at room temperature, but none in those at 32° F. At the end of 7 days germination was noted in all the flasks at 32° F., though the germ tubes were small.

This experiment was repeated. Brown's synthetic medium was used in the place of the apple juice. At room temperature abundant germination occurred in 20 hours, and a few spores had germinated in the solutions at 32° F. in 172 hours.

Spores from 9-day-old cultures were frozen in a 10 per cent sterile applejuice solution and held in an electric refrigerator at 21°-25° F. for 101 hours. The outer portion of the ice was melted quickly and the ice cake washed several times with sterile distilled water to remove any spores from the sides of the flask that were not frozen in the ice. The flasks were held at room temperature. Examination of the solution 36 hours after melting showed germination of the spores, the percentage being nearly as great as in the check held at room temperature.

THE EFFECT OF CLEANING SOLUTIONS ON BLUE-MOLD SPORES AT DIFFERENT TEMPERATURES

The commercial practice is to heat the washing tanks to 100-110° F. during operation and then to shut off the heat during the night. It has been observed that the temperatures of these tanks drop during the night from the initial temperature of 100 to 110° F. at 6:00 p. m. to 70 to 85° F. at 4:30 a.m., depending on the exposure of the machines and the temperature of the air. The heat is turned on again in the early morning, bringing the solution to the operating temperature again in time to begin operations at 7:00 a.m. One would suppose from the work reported in this paper that these conditions would greatly reduce the spore load of the tanks. In order to test this supposition bottles of the treating solution were taken from a machine in Wenatchee, Washington, employing 1 per cent acid and a machine using sodium carbonate-trisodium phosphate cleaner (300 lb. to 250 gal.). Samples were taken at the end of the day's run, before the sediment had settled. On the following day, before beginning the test, these solutions were incubated at 110° F. for several hours to stabilize their temperature. The solutions were inoculated with a suspension of blue-mold spores and plates made in triplicate, 1-300 dilution being used for the acid and 1-18,000 for the alkaline wash (to reduce the inhibiting action of the cleaner). They were then returned to the incubator and the temperature lowered gradually to 72° F. in 111 hours, a thermograph record being kept. Plates were made again at the end of that period (Table 7).

TABLE 7.—Effect of the overnight stand on the number of viable blue-mold spores per cubic centimeter in washing solutions

Time	Temperature	Hydrochl	oric acid		rbonate-tri- phosphate
		Bottle 1	Bottle 2	Bottle 1	Bottle 2
11:30 a.m	110° F.	258,000	468,000	67,500	70,600
11:00 p.m	72° F.	30,000	18,000	3,600	200

Representative plates from the series presented by table 7, showing the number of viable blue-mold spores in the washing solutions before and after the 11½-hour stand, are shown in figure 1.

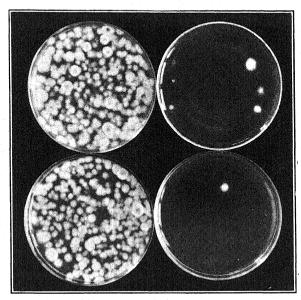


Fig. 1. Cultures from 1 per cent hydrochloric acid cleaner before and after the 11½-hour stand at a temperature gradually lowering from 110° F. to 72° F., showing the decrease in the number of blue-mold colonies.

In this experiment the time and temperature factors were taken at conservative levels. The time occupied by the fall of the temperature and its restoration to the degree used in cleaning is usually 13 hours; in this experiment it was lowered for $11\frac{1}{2}$ hours. In commercial operations the temperature drops to 70–85° F. by 4:30 a. m. and rises to 100–110° F. by 7:00 a. m.; this rise was omitted.

It is believed that the blue-mold spores will nearly all be killed during the overnight stand under the present practice of heating the washing solutions and that formaldehyde treatments, the daily changing of solutions, or other measures aiming at reduction of spore load are unnecessary. It should be noted that this test shows a close correlation of the effect of commercial solutions and the laboratory-test solutions as presented in the following experiments.

Hydrochloric Acid Cleaner. The effect of hydrochloric acid on bluemold spores at the maximum strength of commercial cleaning and at various temperatures was determined (Table 8). The 2 series at room temperature

TABLE 8.—Number of viable spores of blue mold per cubic centimeter obtained at intervals from spore suspensions in 3 per cent HCl at different temperatures

		emperature st series		
The same of the sa	Water sheek	3	% commercial ac	eid
Time	Water check	Flask 1	Flask 2	Flask 3
0 hours 6 '' later 12 '' '' 24 '' '' 36 '' '' 48 '' ''	218 297 226 306 282 235	192 312 127 201 96 16	211 289 225 196 102 14	224 276 212 227 127 32
	Seco	nd series		
0 hours 6 '' later 12 '' '' 24 '' '' 36 '' '' 48 '' ''	1,370 1,706 1,050 1,408 1,243 1,506	1,143 2,023 1,333 1,073 640 226	1,696 1,773 1,770 1,046 510 163	1,496 1,973 1,510 1,143 536 166
	90° F. te	emperature		
0 hours 1 hour later 6 hours '' 12 '' '' 24 '' '' 48 '' '' 72 '' ''	4,120 3,920 3,816 4,053 4,220 4,396 4,330	4,163 4,480 3,063 1,726 393 10	4,500 4,433 2,916 1,793 266 20 0	4,036 4,043 3,086 1,736 130 10
	100° F. t	temperature		
0 hours	2,653 2,343 1,990 1,893 3 0	2,080 2,663 73 0 0	2,713 2,900 40 0 0 0	2,263 1,970 16 0 0
	110° F. t	emperature		
0 hours 1 hour later 2 hours '' 3 '' '' 4 '' '' 5 '' '' 6 '' '' 7 '' '' 8 '' '' 10 '' '' 12 '' '' 24 '' '' 48 '' '' 72 '' ''	4,190 2,866 1,870 2,083 1,883 1,503 1,253 1,370 1,190 13 0	5,290 1,996 143 10 0 0 0 0 0 0 0 0 0	5,720 2,970 1,666 750 6 0 0 0 0 0	4,830 1,886 596 26 0 0 0 0 0 0 0 0

are identical, except for the greater spore concentration in the second. In the flasks with heavy spore loads an average of 12.8 per cent of the spores were viable at the end of 48 hours' exposure as compared to 9.5 per cent in the flasks with the lower concentrations. This is in agreement with the findings of Henderson-Smith (6) and is also observed in the other series to a lesser degree. As it was felt that the variations caused by the factor of unequal spore loads were slight, extreme concentrations of spores were used to lessen error in counting.

Commercial HCl exerted a marked toxic effect in 48 hours at room temperature as compared to water checks, which showed no reduction in numbers. The spores were all killed in the acid solution in 72 hours at 90° F. as contrasted to the water check in which none were killed. The spores were all killed in 12 hours at 100° F. as compared to 48 hours for the water check. The spores were all killed in 5 hours at 110° F. in the acid solution as compared to 12 hours for the water check.

Sodium Carbonate-Trisodium Phosphate Cleaner. The effect of this cleaner on blue-mold spores was tested in the same manner as was the acid solution (Table 9).

The sodium carbonate-trisodium phosphate cleaner exerted a marked toxic effect in 72 hours at room temperature as compared to water checks, which showed no killing. The spores were all killed in 24 hours at 90° F. and the water check was not reduced in 72 hours. In flasks held at 100° F. the spores were all killed in 12 hours as contrasted to 48 hours required by the water check. Spores were all killed in 7 hours at 110° F. as contrasted to a few remaining viable in the water check after 12 hours.

Sodium Carbonate-Borax Cleaner. Difficulty in testing this cleaner was encountered at first, due to its growth-inhibiting properties when transferred from the 1 to 10-dilution tube to the poured plate. Colony formation was irregular in time of appearance and in size as contrasted to water checks.

The use of medium of pH 4.5 gave the same results as ordinary medium of pH 6.2 in tests with the cleaner, with water checks, and with the sodium carbonate and borax separately in strengths at which they occurred in the cleaner. It was found that the borax constituent at strengths used in the cleaner exerted little toxic or inhibiting action, the effect of the cleaner being due to its content of sodium carbonate. The inhibiting effect of the cleaner was due to some property of the sodium carbonate not altered by reduction of its active alkalinity.

The technique used in testing this cleaner was correspondingly altered to reduce this inhibiting action. A flask containing 300 cc. sterile distilled

TABLE 9.—Number of viable spores of blue mold per cubic centimeter obtained at intervals from spore suspensions in sodium carbonate-trisodium phosphate cleaner, 60 lbs. per 100 gal., at different temperatures

	Room te	mperature		
Time	Water check	Sodium carbonate-trisodium phosphate		
Time	water check	Flask 1	Flask 2	Flask 3
0 hours	3,416	4,523	3,253	3,580
1 hour later	3,166	3,450	3,526	3,430
6 hours ''	3,250	2,960	2,980	2,940
12 "	3,003	2,610	2,380	2,263
05 11 11	3,146	1,996	1,973	1,770
40		1,310	1,226	1,073
90	3,133			
10	3,206	596	526	30
72 " "	3,170	260	60	223
	90° F. t	emperature		
0 hours	3,983	3,766	2,866	3,803
1 hour later	3,966	3,250	3,203	3,153
6 hours ''	4,580	1,866	1,016	1,393
12 '' ''	4,016	26	320	46
04 (6 (6	4,153	0	0	0
00 (/ (/		0	ő	0
50	3,966			
10	3,703	0	0	0
72 '' ''	4,230	0	0	0
	100° F.	temperature		
0 hours	3,396	3,450	3,686	3,040
1 hour later	3,423	2,273	2,060	1,743
0 1 ((2,380	3	2,000	70
	2,000			
A. i.e.	2,390	0	0	0
4 T	636	0	0	0
	6	0	0	0
48 '' ''	0	0	0	0
72 " " …	0	0	0	0
	110° F.	tem perature		
0 hours	3,890	3,483	3,953	3,850
1 hour later	2,676	2,533	2,623	3,586
2 hours ''	976	213	60	1,846
3 11 11	606	30	0	
1 11 11				600
T	520	10	6	150
	380	10	3	16
6 '' ''	530	0	0	3
7	286	0	0	0
8 44 44	126	0	0	0
10 " "	20	0	0	ő
12 " "	1	0	ŏ	ŏ
	T		U	1 0

TABLE 10.—Number of viable spores of blue mold per cubic centimeter obtained at intervals from spore suspensions in sodium carbonate-borax cleaner, $1\frac{1}{2}$ lbs. per gal., at different temperatures

	Room ter	nperature		
		Sod	ium carbonate-b	orax
Time	Water check -	Flask 1	Flask 2	Flask 3
0 hours 12 '' later	48,100 53,800	40,900 12,400	44,100 14,900	41,700 12,600
24 '' '' 36 '' '' 48 '' ''	49,100 41,600	13,000 7,800	8,400 14,300	9,200 19,700
60 "	46,800 46,600	3,800 4,600	6,600 3,200	5,100 2,800
72	48,500 43,800 52,900	$1,200 \\ 800 \\ 100$	1,400 1,600 300	1,700 1,100 500
	90° F. te	mperature		
0 hours	42,400	30,200	30,900	41,000
12 '' later	46,500 37,000	29,700 23,800 14,900	26,200 25,300 13,600	27,900 24,600 15,800
48 " "	39,900 43,100 33,900	7,300 4,500	7,200 3,000	6,200 2,800
72 '' '' 84 '' ''	37,800 • 44,200	2,200	1,500	0 0
96 " "	45,500	0	0	0
	100° F. t	emperature		
0 hours 12 '' later	31,400 22,200	33,200 22,600	32,900 21,700	34,000 25,500
24 " " 30 " "	4,500 900	7,800 1,300	2,600 2,100	6,400 2,000
36 '' ''	0 300	700 100	300	600
60 "	100	0	Ů O	ŏ
	110° F. t	emperature		
0 hours	44,700	24,100	25,400 22,600	29,400 23,400
1 hour later 2 hours ''	44,700 25,700 26,400	24,100 26,000 21,900	22,600 20,200	23,400 19,600
1 hour later 2 hours '' 3 '' '' 4 '' '' ''	44,700 25,700 26,400 29,200 27,300	24,100 26,000 21,900 19,700 17,800	22,600 20,200 18,700 19,300	23,400 19,600 24,500 23,700
1 hour later	44,700 25,700 26,400 29,200	24,100 26,000 21,900 19,700	22,600 20,200 18,700	23,400 19,600 24,500

water was used instead of a 9-cc. water blank, and 1 cc. of the test solution was transferred to it. After being shaken 5 minutes, 1 cc. of this 1 to 300 dilution was transferred to a Petri dish and 2 per cent dextrose potato-agar poured over it. Greater spore concentrations were used than in other series due to the greater dilution employed. In 10 plates made from each of 3 flasks treated in the above manner there was no more variation than from a similar series run from a water check. Table 10 shows the action of this cleaner on spores of blue mold.

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This cleaner exerted a slight toxic effect in 48 hours at room temperature and a considerable toxic action in 96 hours as compared to the water check, in which the spores were not killed in the longer period. The spores were all killed in 84 hours at 90° F. as contrasted to no reduction in the water check in 96 hours. In flasks held at 100° F. the spores were killed in 60 hours, nearly the same as in the water check. The number of viable spores was considerably reduced in 11 hours at 110° F., nearly the same as in the water check.

THE EFFECT OF SODIUM HYPOCHLORITE ON BLUE-MOLD SPORES

A great deal of the spore contamination of the washing tank is obtained from the air of the packing house and from the washing of the fruit. Dirty picking boxes that have enormous loads of blue-mold spores are frequently used. Huber's found from 127,860 (5 per cent blue mold, equivalent to 6,393 spores) to 531,200 colonies (30 per cent blue mold, equivalent to 159,360 spores) per square inch on the inside surfaces of the bottom boards of dirty boxes. These spores scatter onto the fruit and hence get into the washing tank or are blown into the air and settle on equipment. It has been found that the condensed steam from the heated washing solutions would enable blue mold to grow on the near-by overhead packing-house timbers, forming striking incrustations. Spores from these sources will increase the spore load of the washing tank directly or indirectly and will settle on fruit after washing. As this will increase the percentage of decay, reduction of these sources of contamination is desirable.

Yesair (13) found that *Penicillium expansum* was one of the most common molds on the walls and equipment of meat-packing houses. He used sodium hypochlorite solutions of different strengths for 5- to 10-minute periods successfully in reducing spore load of these places.

A commercial sodium hypochlorite solution containing 3.4 per cent available chlorine was tested for its effect on the viability of blue-mold spores.

⁸ Huber, G. A. Fungous flora of the normal apple: the sources of contamination and spore load. Unpubl. thesis, Washington State College, 1931.

⁹ Chlorosan (now called Westochlor), furnished by the West Disinfecting Co., Seattle, Washington.

In 6 trials all of the spores in a concentrated suspension in a solution of 3 oz. of this compound per gallon were killed in 15 minutes and at a strength of 0.1 oz. per gallon in 3 hours.

Blocks 1 in. square were cut from apple-box sides and dipped in a suspension of blue-mold spores. Washings were made from some of these by the method described by Huber (7) for determining the spore load of apples and the average spore load determined by plating out portions of the wash water. Other blocks were sprayed with the commercial sodium hypochlorite solution (3 oz. per gallon) and washings made to determine the load of viable spores. Blocks averaged 118,400 viable spores per square inch after dipping in the suspension. Four and a half hours after being sprayed with the sodium hypochlorite solution other blocks had an average of 12,700 viable spores per square inch, or about a 90 per cent kill. As the boxes from which these blocks were taken were slightly dirty some free organic matter was present. Thus, spraying with sodium hypochlorite solutions would be an easy and reasonably effective way of reducing the spore load of packing-house equipment.

DISCUSSION

The practice of washing apples for removal of arsenical residue is now well established in the principal apple districts. Hydrochloric acid cleaner is the one principally used, but alkaline washes containing considerable amounts of sodium carbonate are also widely employed. It has been repeatedly shown that the washing tank is one of the main sources of the spore load of apples. At present these solutions are used without special consideration of decay prevention because no solutions have been found that are of practical value.

It has been shown in this paper that, contrary to the previous reports on Penicillium species, temperature of the solution within the ranges employed does exert a lethal effect in itself on spores of *Penicillium expansum*. This action was markedly increased by addition of the washing compound. The action of both temperature and cleaners is summarized in table 11.

The lethal action of the sodium carbonate-borax cleaner was shown to be due to the constituent sodium carbonate, and the borax in the amounts used was only slightly toxic.

It has been stated by other investigators that the washing solutions are not sufficiently toxic to blue-mold spores to be of any value in reducing the load of viable spores under the conditions used. It has also been stated that the temperature factor is unimportant. In this paper it has been shown that the temperature and the toxicity of the cleaning solution are important factors in the lethal action of cleaning solutions. The action is of a slow type, and no action would occur on spores on apples during their

TABLE 11.—Summary of the effect of cleaning solutions and of water at different temperatures on blue-mold spores

			Washing solution	
Tempera- ture	Water check	Hydrochloric acid	Sodium carbon- ate-trisodium phosphate	Sodium carbon- ate-borax
Room	None killed in 96 hours	87-90% killed in 48 hours	89% killed in 48 hours 95% killed in 72 hours	87% killed in 48 hours; 90% killed in 72 hours; 97% killed in 96 hours
90° F.	None killed in 96 hours	All killed in 72 hours	All killed in 24 hours	All killed in 72- 84 hours
100° F	All killed in 48 hours	All killed in 12 hours	All killed in 12 hours	All killed in 48- 60 hours
110° F	All killed in 12 hours	All killed in 4-5 hours	All killed in 6-7 hours	86% killed in 11 hours

brief exposure in washing. For that reason the claims of the manufacturers that their cleaners will effectively control blue mold are unjustified. The cleaners as used at present, however, will keep the tanks reduced to a concentration of viable spores whose maximum will be that accumulated in a day's run.

Tests with samples taken from washing solutions at the end of a day's run showed a 93.3 per cent and 97.4 per cent kill of the spores in acid and sodium carbonate-trisodium phosphate cleaners, respectively, after $11\frac{1}{2}$ hours' exposure at a temperature gradually decreasing from 110° to 72° F. It is not probable that any special means of reduction of spore load of the washing tanks from day to day is needed at present.

A close correlation existed between the laboratory tests and the conditions actually occurring in the washing tanks.

The lethal action on young and old spores was shown not to vary so markedly with their age in the case of high temperatures as with the toxicity of cleaners. The action of hydrochloric acid and sodium hypochlorite solution was shown to be inversely related to spore age.

The lethal effect of the cleaners was generally found to vary inversely with the spore concentration when other conditions were equal. The variations was so slight, however, that it is felt that the number of spores is not a significant factor in their resistance to either temperature or toxic agents in packing-house operations.

It was found that spores germinated in 7 days at 32° F. in 25, 50, and 100 per cent apple juice and in Brown's synthetic medium. Spores frozen in 10 per cent apple juice and held at 21°-25° F. for 4 days germinated readily on being returned to room temperature. It is probable that infection of apples can occur at 32° F. from spores germinating in storage and that infection has not always occurred previous to the drop in temperature to 32° F.

Sodium hypochlorite solutions have been found to have high toxicity for *Penicillium expansum* spores and may be used as a spray in packing houses to reduce spore contamination. The effect of these solutions when applied directly to the fruit is being investigated.

SUMMARY

- 1. The washing tank is one of the main sources of contamination of apples with blue mold, and the reduction of its load of viable spores directly or indirectly is desirable.
- 2. Age is of less importance in resistance of *Penicillium expansum* spores to high temperatures than to toxic chemicals. Old spores were consistently more resistant to the toxic agents tested than young ones.
- 3. Spore concentration in washing solutions is not a significant factor in resistance of the spores to either the temperature factor or to toxic agents in washing tanks.
- 4. The toxic action of the principal cleaning solutions now used is of no greater importance than that of the temperatures at which they are used, although the addition of these to the water decreases the time required for a complete kill.
- 5. The toxicity of the sodium carbonate-borax cleaner is due to the constituent sodium carbonate, and this cleaner has marked inhibitory action in high dilution.
- 6. Practically all of the blue-mold spores contained in the cleaning solutions are killed during the overnight stand, though the tanks are not being heated during that time.
- 7. Special means of reducing the spore load of the washing tanks from day to day are unnecessary under present conditions of operation.
- 8. Blue-mold spores failed to germinate at room temperature or 90° F. in sterile distilled or tap water. They germinated in 12 hours in 2 per cent apple-juice solution at room temperature but did not in 14 days at 90° F.
- 9. Germination of blue-mold spores occurred in sterile apple juice at 32° F. in 7 days.
- 10. Spores held at 21°-25° F. in 10 per cent apple juice for 101 hours germinated readily on being returned to room temperature.

11. Sodium hypochlorite solutions are very toxic to *P. expansum* spores and could be used to advantage for spraying packing-house equipment to reduce spore contamination.

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A PRELIMINARY STUDY ON THE USE OF INDELIBLE PENCILS AS INDICATORS OF THE MOISTURE CONTENT OF WOOD

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When an indelible (anilin) pencil is drawn across a moist or wet surface the mark is of a very different color from that made on a dry surface. The common purple pencils of this type, for example, leave a somewhat glossy pale gray mark on a dry surface, but a dull and more deeply colored purple mark on a wet surface. This generally known property of such pencils suggested a series of studies looking toward the correlation of the streak color with the moisture content of wood. These studies were conducted at the School of Forestry of the University of Idaho during the academic year of 1928–29.

Following inquiries, a number of pencil manufacturers submitted for testing sample pencils of various colors. Each of these pencils was given an arbitrary number for convenience in keeping records. The 4 used principally in the investigation are listed in table 1.

TABLE 1.—List of principal indelible pencils used, with maker's name and designation, and colors of streaks

			Color of streak				
Pencil No.	Maker	Maker's designation	On pal	e wood	Onwhite writing paper		
		uosignation	Dry	Wetted and dried	Dry	Wetted and dried	
8	Joseph Dixon Crucible Co. American	Eldorado, master copying, 213 soft	Neutral gray	Blackish violet	Violet gray	Dark violet	
13	Lead Pen- cil Co.	A special indelible	Rose doree	Begonia rose	Carmine	Begonia rose	
15	Koh-i-noor Pencil Co.	Mephisto copying, 73F.	Neuvider green	Vivid green	Dark viridian green	Vivid green	
20	Blaisdell Pencil Co.	Super-thin, 754	Light orange yellow	Light cadmium	Deep chrome	Strontian yellow	

The color streaks of these 4 pencils can be described in general terms as purple, red, green, and yellow, respectively. More precise designations for the streaks on pale-colored wood (sapwood of western white pine (*Pinus monticola* D. Don.)) and on white writing paper are given in the last 4 columns of table 1. These are based on comparisons with the plates in

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R. Ridgway's "Color Standards and Color Nomenclature" (1912). For the "wet" color in the case of the wood the surface was first moistened by sponging, while on the paper patches of the dry color were wetted with a sponge.

In the earlier stages of the experiments it was thought that a set of pencils might be found that would show marked color changes due to solution of the coloring material and corresponding to diverse moisture contents of the wood, and the several pencils were tested with this in mind. Later, when considerable preliminary work had been disposed of, a systematic test was made with about 100 blocks of western white pine. In this series 48 different moisture contents were represented, ranging from 16 per cent to 87 per cent, on the oven-dry basis. The 4 pencils above listed were tested on each block. When the test blocks, after oven drying, were arranged in order of their moisture content prior to drying it became evident that solution of the streak could not be clearly correlated with any definite moisture content of the test material. On the contrary, there was for each pencil a whole series of streaks of slightly different color tone referable to small differences in moisture content of the test blocks. The differences in tone between any 2 adjacent blocks were scarcely distinguishable, although the differences between the streaks on blocks containing, respectively, 19 and 69 per cent moisture, for instance, were quite pronounced.

After this, these studies were directed toward preparing sets of standards with the several pencils and with various species of wood and testing out the utility of such standards by actual trial. Most of the work was done with streaks of the 4 pencils listed in table 1 on blocks of western-white-pine sapwood, and the following discourse has to do primarily with standards and test blocks of this material. The results from tests with other pencils and other wood species are not numerous enough to warrant detailed presentation.

In the main, small blocks about 1.3 cm. square and 2.5 cm. long were used in these studies. These blocks were placed 10 in a Stender dish and an amount of water was added sufficient to give a calculated average moisture content. This amount was based on the air-dry weight of the group of blocks and the moisture content, ascertained by drying, for sample blocks from the same stick. Sometimes an unmeasured amount of water was added. The Stender dishes were about 37 mm. high and 76 mm. in diameter, inside, and the covers had a ground groove fitting tightly against the ground edge of the dish. Generally, the dishes were left standing a day after adding the water and covering. 'They were then subjected in an autoclave to a temperature of 98° C. for an hour and finally left standing another day before the tests were made. It was found that this treatment resulted in very uniform distribution of the moisture.

In making a test the block was first weighed and then split open and streaks quickly made from the center to the end, 2 streaks for each pencil (using different sides of the lead), 2 pencils to each freshly exposed surface. An effort was made to use uniform pressure in making these streaks. After drawing the streaks the pencil points were dried by rubbing on a piece of paper. The blocks were oven-dried and the moisture content at the time of making the streak computed. These blocks, suitably mounted on cards, then served as standards in subsequent studies.

With such standards, some 300 estimates were made of the moisture content of blocks and the actual moisture content was then computed. Some results of such tests will suggest the possibilities of the pencil-streak method.

Of 82 estimates made with standard streaks of pencil No. 8, 52 per cent were within 5 points (above or below) the actual moisture content in percentage, 72 per cent within 10 points. The following figures give results for the 4 pencils here considered:

Pencil No.	Correct estimates	Estimates within 5 points	Estimates within 10 points	Total tests
8	Per ct.	Per ct. 52	Per ct. 72	82
13	5	54	71	82
15	6	58	74	81
20	7	57	74	81

While these percentages can not be considered acceptably close for all types of work requiring a knowledge of moisture content, yet they might, for example, prove to be close enough in brush-burning work. At any rate, they do suggest the possibilities of the method. At the present stage of the study it can not be stated with assurance just why the errors should be so large. In some cases they are perhaps attributable to an insufficient set of standards. And undoubtedly the matter of practice is to be considered an important factor. Possibly streaks of some other pencil than the 4 studied most intensively, namely, Nos. 8, 13, 15, and 20, would give more satisfactory standards than these. The standards used were kept in covered cardboard boxes after drying, except for the short time during which comparisons were being made, and exposure to direct sunlight was always avoided.

The method thus far developed by these tests gives promise of utility in certain ways, especially where it is desirable to study the distribution of moisture in wood with pronounced differences in moisture content. An example will serve to illustrate some of the possibilities of this method.

An air-dry block of western-white-pine heartwood 20 cm. high and 5 cm. thick was kept standing on end in water 3 or 4 cm. deep, the water being brought up to the original level from time to time. At the end of $7\frac{1}{2}$ weeks, the outside was tested with 3 indelible pencils, and it was found that at about the level of the water surface, 4.5 cm., and abrupt color change took place from dry colors (corresponding to less than 16 per cent moisture content) to wet colors (corresponding to more than 87 per cent moisture content). On a freshly split surface roughly parallel to the outside surface and 1 cm. from it, the change occurred 2.5 cm. from the bottom. That is to say, in $7\frac{1}{2}$ weeks with the water in which it was immersed usually at least 3 cm. deep the penetration inside was to a lesser height than the depth of the water. On the outside of the block the moisture had risen slightly above the water level.

After 3 months the water was allowed to evaporate without being replenished. About 2 or 3 weeks after the water was gone, pencil tests were again made. On the outside at this time and 1 cm. from the edge the moisture content, as shown by the streak colors, was less than 16 per cent. On a face 2 cm. from the edge there was a pronounced moist area about 3 cm. long and 2.5 cm. wide. Outside of this area, the moisture content was apparently less than 16 per cent. The transition from dry to wet wood was rather abrupt, the moisture content of the outer portion of the moist region being apparently at least 70 per cent. At the center the moisture content was probably over 90 per cent. The moist area extended to the bottom of the block and was situated in the middle of the section.

E. E. Hubert, of the Idaho School of Forestry, observed a marked color change occurring at the point where the moisture content of a block began to inhibit the growth of a blue-stain fungus. At this time, no standards were available for interpretation of the percentage moisture contents.

On the whole, it seems likely that this method, when perfected, would in some cases give more satisfactory information about the moisture in a piece of wood than that obtainable from the ordinary method of weighing and oven drying. In this latter method the average moisture content of the test piece is ascertained. If, for instance, a disk be cut from a stick in a brush pile the average moisture content of this can readily be ascertained. A more nearly exact picture would, of course, be given by making separate tests of blocks from the upper and lower parts of the stick as it lay in the pile. But, if the moisture distribution could be ascertained by making pencil streaks on freshly exposed surfaces of such wood, we ought to obtain thereby a far better understanding of the moisture conditions in such a stick. It seems likely that the method could be used also in investigating in detail moisture conditions in various parts of a pile. Such precise

information might very well prove of the highest significance in interpreting the activities of fungi or insects in brush-pile wood and in laying plans for brush-burning activities. A further application that may be envisioned is in forest-fire studies, where it is desirable to ascertain, as a measure of inflammability, the moisture content in various parts of logs and branches lying on the ground.

Possibly the pencil method could also be substituted for the oven-drying method in making studies of the moisture content of test material in which the moisture distribution is essentially uniform. It is, however, to be kept in mind that there are still several problems to be solved in connection with the method here discussed before it can be used except upon rather a limited scale by expert investigators. Some of these problems will now be briefly discussed.

In the first place, it seems unquestionable that the color tone will vary with the color of the wood upon which the streak is drawn. It is possible, too, that the tone may vary between streaks made on wood of the same color but of different species. It may be that the color will not be constant for pencils from different batches of the same brand and style number.

Another problem has to do with the question of permanence of standards. A few preliminary tests were made to ascertain the effect of strong sunlight upon the colors. For this purpose a series of test blocks was shellacked and mounted on a board, "Superior Pure Shellac, White," manufactured by the Glidden Company (of Cleveland) being used. These blocks (of western-white-pine sapwood) had been oven dried after marking with pencils Nos. 8, 13, 15, and 20, and the moisture content ascertained. One half of each streak on the test blocks was covered with black paper. They were then placed in a south window to receive full sunlight and left for a few days.

Examination of these test blocks disclosed that in most cases the colors had completely faded out. At 6 per cent moisture content and also at 84, 90, 99, 105, 119, and 125 per cent in the case of No. 15, part of the streak was still discernible, but the color was much paler than on the part of the block that had been shaded. At 6 per cent in the case of No. 20 the streak was orange rather than yellow. In the case of No. 8 streaks, the color was not appreciably faded at 6 and at 15 per cent. The other streaks had changed from purple to gray (black at 125 per cent), but graduated differences in intensity were still obvious.

On the whole, the study of this phase of the subject indicates that the streaks of Nos. 13, 15, and 20 have no permanency when exposed to bright sunlight under the conditions of the test, and the streaks of No. 8 are modified to such an extent that they would be of doubtful value as standards.

Another test was directed toward ascertaining the possibility of water-proofing color standards with shellac, so that they could be handled without risk of alteration due to accidental access of moisture. Streaks were made on dry sapwood of western white pine and these then shellacked. Streaks of some of the pencils were found to run notably, and the color of the streak was appreciably altered by the shellacking in a considerable number of cases.

It is to be noted that there was a certain amount of color change, also, incident to baking in oven drying to ascertain the moisture content for use as a standard.

All things considered, it would be preferable not to use the pencil streaks themselves for standards but to have permanent, durable standards prepared to match the streak color corresponding to each percentage index (and for each wood species and test pencil it is desired to use). It is also to be remembered that any system depending upon the matching of colors is somewhat restricted in availability because of the inadequacy of certain artificial lights for the purpose and because of the difference in ability of different persons to distinguish closely allied hues, shades, and tints. Perhaps the pencil manufacturers would be interested in developing such standards.

SUMMARY

This paper describes some preliminary tests on the use of indelible pencil streaks for ascertaining the moisture content of wood. This method apparently offers a promising means of studying the moisture distribution in a test sample of wood, at least when pronounced differences in moisture content are found in different parts of the test piece. For investigating the moisture in wood where the distribution is irregular, as in a stick from a brush pile or a fallen log, it seems likely that this method, when perfected and in the hands of a skilled technician, would give more satisfactory information than that obtainable by the ordinary methods of weighing and oven drying, which, of course, give only the average moisture content of the test piece. Possibly the pencil method could also be substituted for the oven-drying method in making studies of the moisture content of test material in which the moisture distribution is essentially uniform. These studies have, however, not yet reached a stage where this seems feasible.

A number of problems connected with this method are still to be solved. These have to do mainly with the preparation of usable permanent standards.

CARMEL, CALIF.

MILD STREAK OF BLACK RASPBERRIES¹

L. M. COOLEY
(Accepted for publication March 7, 1932)

The eastern blue-stem or streak virous disease of black raspberries was first described by Wilcox in 1923 (6) from Cuyahoga County, Ohio. Since then, for this State as a whole, streak has been found to be the most prevalent of the virous diseases of black raspberries. In Ohio, the symptoms have usually been distinct, typical, and uniform and have agreed closely with Wilcox's original description.

Following some field experiences with a peculiar streak type of disease found in a few black raspberry plantings in northeastern Ohio in 1927 and 1928, the writer decided that there were two separate kinds of streak in black raspberries. Subsequent observations and studies, made each season since then, have tended to confirm this belief. The name "mild streak" was given to the new type. The older and better-known form was designated "severe streak." These names seemed descriptive of the differences in the effects produced on host plants by the two forms. In such a nomenclature, the name severe streak is synonymous with eastern bluestem or streak as described originally by Wilcox.

This conception of two separate, but quite similar, diseases seems to explain the confusion evident in the literature as to the exact symptoms of streak and their constancy and the identification of the disease in different berry-growing districts in eastern United States. Bennett (1) found that the mottling of foliage and the streaking of canes tended to be indistinct under Michigan conditions. In 1927, Rankin (4, p. 48) described a disease type in black raspberries in New York that showed some of the symptoms of streak, but "the blue marks on the canes described for streak rarely showed and the progressive later stages for streak were not evident." It now seems probable that these investigators were working, for the most part, with the mild type of streak. In a recent classification of raspberry viruses, Rankin (5) adopted the conception presented here, listed mild streak as a separate virous disease, and gave a brief description of its symptoms.

Mild streak is a disease of cultivated varieties of the black raspberry, *Rubus occidentalis* L., and is believed to be caused by a virus. The malady has not been recognized on any other host.

¹ Published with the consent of the Director of the Ohio Agricultural Experiment Station.

SYMPTOMS

Black-raspberry plants affected with mild streak are never stunted seriously; if heavily fertilized, they may make remarkable growth.

The most constant foliage symptom exhibited by a diseased plant is the characteristic hooking, recurving, and twisting of the midribs of leaflets at the tips of the new canes (turions). Usually, the midribs of all other leaves also curl slightly and arch downward, giving the entire plant a drooped or faintly "rosetted" appearance. The foliage, in general, also displays a green color just a shade darker than normal. The old leaves are never blotched or mottled, although they may develop a faint lightening of color along the main veins.

Turions may or may not be streaked at their bases. The longitudinal streaks, if present, are narrow (½ mm. or less in width). They are steel-blue and gray in color (approximating Hathi gray of Ridgway's color chart) and are obscured by the translucent epidermis and cuticle. Such streaks may be present also on the stems of fruiting laterals, on petioles, and on flower or fruit pedicels.

The most distinctive and diagnostic single symptom of mild streak is the manner in which the fruit is affected. The drupelets on individual berries develop unevenly. This effect can be noted from the time of flowering until the berries are gone. When the normal time for the ripening of the fruits is reached, on the same berry some drupelets will be hard and unripe (some red, others green), a few will be ripe but of disagreeable flavor, and still others will have ripened prematurely and decayed. As a consequence, the berries on mild streak plants are "piebald," small, seedy, and distasteful. A black-raspberry plant with mild streak may live for several years without a marked reduction in vigor and yet never bear a crop of marketable fruit. Because of this particular affection of the fruit, the mild form of streak occasions more direct economic loss per plant infected than does severe streak.

Symptoms of mild streak are expressed most noticeably during that period of the year when temperatures are highest. In Ohio, the disease may be distinguished clearly in July and August. In some seasons the symptoms are more pronounced than others, correlating with the general temperature range. Plants that are given good culture and are growing rapidly express symptoms (other than stunting) more markedly than neglected, slow-growing ones. Although the phenomenon has not yet been witnessed by the writer, it is considered possible that the mild-streak virus may be resident in a black-raspberry plant and remain completely masked throughout an entire season.

COMPARISON OF MILD STREAK AND SEVERE STREAK

Several of the symptoms of mild streak indicate a close relationship of this disease with severe streak. The same typical recurving and hooking of the midribs of tip leaflets of the turions is a symptom common to both diseases and, in both, it is identical in appearance and extent. A general rosetted appearance of affected plants is noticeable with both diseases; but, since the growth is not stunted much by mild streak, the rosetting is less pronounced. Streaks occurring on turion bases, on stems of fruiting laterals, and on petioles are a common symptom, but the streaks themselves are dissimilar.

The two diseases coincide in their seasons of symptom expression and in their temperature responses. Both diseases seem rather specific to black raspberry, though Wilcox (6) cites instances of severe streak on black-berry plants.

Despite these similarities, mild streak may be distinguished from severe streak rather readily by certain symptomatic differences. In the writer's experience in Ohio, a conspicuous and distinctive blotching or gross mottling of the older foliage is present in all fully developed cases of severe streak; this character is absent or very faint in the mild type. The streaks, from which both diseases derive their names, are different: broad, short, and dark blue or brown in the severe; slender, long, light blue-gray in the mild. The differences in host growth are marked: severe streak causes an appreciable and comparatively rapid stunting by shortening the internodes, the final result being a decided depression of longitudinal growth; mild streak produces only a slight stunting, and, under circumstances favorable for host growth, this is not appreciable. The stunting produced by severe streak becomes more severe with each successive season; the slight stunting of mild streak does not seem to be cumulative. The peculiar affection of fruits by mild streak has been described above. In contrast, in the case of severe streak the drupelets of the berries develop uniformly, and, though the fruits are of poor quality, they are marketable.

Mild streak is not likely to be confused with any other of the virous diseases of raspberry. However, leaf hoppers sometimes produce foliage injury deceivingly like that of mild streak. If fruits are present on suspected plants, an examination of them will reveal mild streak. Leaflets that have been attacked by hoppers show an injured brown area on the dorsal surface of their midribs; this usually serves to distinguish hopper injury.

ETIOLOGY

The belief that mild streak is a virous disease is based on field evidence. No pathogenic agency that conceivably could cause the disease has ever been found associated with diseased individuals, and in no case has it been possible to correlate the occurrence of diseased plants with any environmental factor.

Symptoms are expressed by all plant parts aboveground; this would indicate the systemic nature of the disease. The symptoms themselves, especially in their mild resemblance to those of severe streak, are suggestive of a virus, and, as stated above, a direct correlation exists between symptom expression and temperature.

Mild streak recurs in succeeding seasons on plants once affected but does not develop into the severe type. The disease is disseminated by the vegetative propagation of diseased individuals.

Under most conditions, the rate of spread of mild streak is slow, about doubling each season in the plantings observed. But, in vigorous plantings, spread has been seen to take place much more rapidly. Plants adjacent to a diseased individual are apt to become infected, but the disease seemingly may be carried almost as readily to more distant plants. Spread very commonly takes place to the leeward of an infection source. The distribution and rate and time of spread are not only indicative of a virous trouble but also suggestive of an insect vector.

The successful experimental transmission of streak, either mild or severe, has not yet been accomplished. Consequently, evidence of infection of the virous nature of mild streak is lacking. Nor has it been possible to test for separate viruses by inoculation work.

DISTRIBUTION

No specific survey for mild streak has been made in Ohio, but the disease has been found definitely in 12 plantings on 8 different farms, in 5 counties. Of the 12 plantings, 11 were of the Cumberland variety; the other one, Plum Farmer. In 7 of the 12 plantings, severe streak was present also, furnishing opportunities for critical comparisons of symptoms. Six of the 8 farms where the disease has been found were located in the easternmost tier of counties (Columbiana, Mahoning, and Ashtabula). One heavily infected planting was in Cuyahoga County and another in Sandusky County. On the whole, the occurrence of mild streak in Ohio is rather rare in comparison with the severe type.

Bennett (2, p. 16) found streak to be "less common in Michigan [than in Ohio] and as yet [1928] it has not spread very rapidly or widely." He remarked in conversation with the writer that the mild type was much more frequent in Michigan than was the severe one.

"Mild streak is more or less prevalent in central and western New York" and is much more common there than severe streak (Rankin, 5, p. 9).

Judging from the descriptions of symptoms given, Berkeley (3) has reported what seems to be mild streak of black raspberries occurring in two plantings in Ontario, Canada.

VARIETAL SUSCEPTIBILITY

In Ohio and Michigan, the black-raspberry variety Cumberland has been the one most frequently affected with mild streak. One instance in Plum Farmer has been found in Ohio. At Rogers, Ohio, an extensive planting of the variety Improved Kansas has been growing for several years beside a large Cumberland planting that contains numerous individuals affected with mild streak. During the 3 years these plantings have been under observation, the mild streak has spread rather widely within the Cumberland planting, whereas not one of the adjacent Improved Kansas plants has become infected.

In New York the variety Cumberland is grown but little. There, Rankin (5) has reported that mild streak is particularly prevalent in the varieties Ohio and Kansas.

From these rather meager observations it would seem that the relative susceptibility of black-raspberry varieties to mild streak probably corresponds closely with that given by Wilcox (6) for severe streak, and it seems equally true in the case of mild streak that susceptibility is correlated closely with varietal characteristics of growth habit and vigor—the more vigorously growing varieties seeming more susceptible.

STIMMARY

Mild streak is a disease of the black raspberry, Rubus occidentalis L., believed to be caused by a virus. The malady has not been recognized on any other host.

A description of symptoms is given. Symptom expression has been found to be most pronounced during the hot summer months.

The belief that mild streak is a virous disease is based on field evidence. The successful experimental transmission of mild streak has not yet been accomplished and final experimental evidence of infection is lacking.

The distribution, rate, and time of spread of mild streak are not only indicative of a virous trouble but also suggestive of an insect vector.

The names "mild streak" and "severe streak" are suggested to differentiate between what seem to be two separate diseases of similar nature. In this concept, severe streak is considered synonymous with eastern blue stem or streak of the black raspberry, as described originally by Wilcox.

Mild and severe streak are compared as to symptoms, seasons of symptom expression, and host ranges. Several of these symptoms indicate a close

relationship existing between the two diseases; others serve as a diagnostic means of distinguishing between them.

Notes on the known distribution of mild streak and varietal susceptibility to the disease are given.

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NOTABLE OUTBREAKS OF CRANBERRY FRUIT ROTS IN MASSACHUSETTS

NEIL E. STEVENS
(Accepted for publication March 14, 1932)

THE MASSACHUSETTS CRANBERRY CROP OF 1931

Judged by the high standards of excellence prevailing among those who grow and handle Massachusetts cranberries, the crop of 1931 was of unusually poor keeping quality. In the opinion of some observers it was inferior even to the notorious crop of 1914. An idea of the relative amount of loss from decay during recent years may be gained from a comparison of the average amount of decay in storage lots from representative bogs in Plymouth County during the last 4 years (Table 1). The figures given are for the condition on November 15, when the fruit had been in storage approximately 2 months.

The Early Black is the most important variety in Massachusetts and makes up approximately half the total production. The Howes is second in importance and is the standard late variety. Eight to 10 lots were stored for each variety.

TABLE 1.—Average condition of test storage lots of cranberries expressed as percentage of rotten berries, November 15

	Percentage of rotten berries, November 15						
Variety	1928	1929	1930	1931			
Early Black	7.4	11.0	13.1	31.0			
Howes	6.5	9.0	6.7	14.1			
Odd varieties	12.2	18.0	15.6	30.5			

OTHER RECENT CRANBERRY CROPS WITH UNUSUAL LOSSES FROM FRUIT ROTS

Fruit rots of cranberries are, as is well known, caused by various fungi. Since, in general, the fungi concerned are indistinguishable in their effect on the fruits and are controlled by the same methods, they must for practical purposes be studied as a group.

In the case of the cranberry crop of eastern Massachusetts, there exists an unusually complete record of the relative amount of fruit rots for crops from 1912 to date. These are preserved in the reports of a cooperative sales company that handles a large portion of the crop of the state.

The existence of these records, together with weather observations taken in the immediate vicinity and in close proximity to a cranberry bog, made possible the initiation of a study of the relation between the keeping quality of cranberries and prevailing weather conditions. This work, begun in 1922, resulted in an attempt to predict the keeping quality of individual crops. These predictions have been made for 9 years with such success that they have become a part of the regular commercial practice of the region and may be said to have passed the experimental stage.

As this work has been briefly discussed elsewhere¹ and a detailed account is to be published later, no reference is here necessary further than to say that the keeping quality of the crop seems to be correlated with the temperature during May and June and the frequency of rainfall during July and August. In general, it appears that unusually high temperatures during May and June, the period before the blossoms open, tend to be unfavorable to good keeping quality in the crop. Also, especially in the late varieties, unusually frequent rainfalls during July and August, the season during which the fruit is growing, apparently tend to favor an increase in decay. A combination of warm weather in May and June with frequent rainfall during July and August is apparently the most unfavorable condition of all.

The chart (Fig. 1) is designed to show the combined influence of temperature during May and June and frequency of rainfall during July and August. The abscissas represent temperature in May and June, here computed as a summation of day degrees above 50, and the ordinates the number of days with 0.01 in. or more rain during July and August. The heavy lines indicate approximately the normals. The upper right-hand portion thus includes those years having cool springs followed by summers with less than the average number of days with 0.01 in. or more rain and the lower left-hand portion those years having unusually warm springs followed by summers with more than the average number of days with 0.01 in. or more precipitation.

The abundance of fruit rots or, in other words, the keeping quality of the Howes variety each year, is indicated by symbols. Black dots indicate the crops having the largest losses from fruit rots. Circles with cross hatching indicate crops with keeping quality below average. Circles with F indicate fair quality, and unshaded circles, crops with slight losses from fruit rots. A similar chart based on the keeping quality of the Early Black variety shows a somewhat larger number of years above average quality but no other difference.

It will be noted that the 3 very bad years of record, 1914, 1922, and 1931, all fall in the group having warm May and June and more than ordinary number of days rain in July and August, and that the 3 other years

¹ Stevens, N. E. Cranberries used in trial forecasts as to keeping quality. U. S. Dept. Agr. Yearbook 1927: 238-240, 1928 (and earlier papers on the same subject).

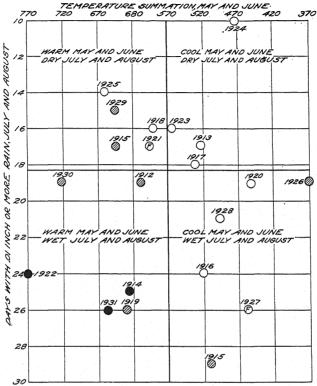


FIG. 1. Temperature during May and June and frequency of rainfall during July and August at East Wareham, Mass., 1912 to 1931, and the relative abundance of fruit rots in cranberries of the Howes variety in the Massachusetts crops of those years. Black dots, exceptionally large losses from fruit rots; circles with cross-hatching, losses from fruit rots larger than normal; circles with F, fair keeping quality; unshaded circles, slight losses from fruit rots.

in this group all had poor keeping quality. On the other hand, in the group having warm May and June but relatively dry July and August, there are 2 poor, 1 fair and 2 good crops, and in the group having a cool spring but frequent rain during July and August, 2 poor, 1 fair, and 3 good years. The 4th group, that having a cool spring and relatively dry July and August, on the other hand, contains thus far only years having crops of very high keeping quality.

EARLY RECORDS OF UNUSUAL LOSS FROM CRANBERRY FRUIT ROTS IN MASSACHUSETTS

Although the systematic study of plant diseases began in this country about 50 years ago and publications in this branch of botany have appeared in constantly increasing volume since 1886 or 1887, there is a surprising

dearth of information regarding the extent and severity of outbreaks of plant diseases, particularly diseases of minor crops. In attempting to trace the history of diseases of many crops, the student is too often driven to searching horticultural and agricultural magazines for chance references. Such records of diseases as exist are frequently in unpublished notes. So rare, indeed, are such records in available literature that almost any authentic record of a disease outbreak prior to 1900 deserves publication for its possible value to pathologists of to-day. The Plant Disease Survey project of the Bureau of Plant Industry was organized partly to provide a centralized unit where such records of diseased outbreaks could be recorded and made permanently available. Its records are, however, still fragmentary, and, of course, largely recent.

The interest aroused by the unusual amount of decay in the cranberry crop of 1931 led, first, to a search for records of previous crops of equally poor keeping quality and, second, to the study of weather records to determine, if possible, the frequency of the occurrence of weather that might have been expected to produce poor keeping quality. For obvious reasons unusual abundance of diseases is more likely to be made a matter of record than unusual freedom from disease. That is, a crop of poor quality is more likely to be recorded than one of high quality.

Such records of unusual losses from rot of cranberries prior to 1912 as could be found are in an early paper by Halsted and in the Proceedings of the American Cranberry Growers' Association, which, although its head-quarters and most of its officers were in New Jersey, at that time numbered among its members growers from other States and maintained a lively interest in the crops of those states. From the records of this Association, it is evident that the crop of 1889 was of exceptionally poor keeping quality, both in New Jersey and Massachusetts, much like the more recent years of 1914 and 1931. 1889 was the year in which Byron Halsted was appointed botanist and horticulturist at the New Jersey Agricultural Experiment Station and took up a study of cranberry "scald." Early in his work he circularized a number of cranberry growers in Massachusetts and New Jersey and from his published record² it appears that the cranberry crop was considered of poor keeping quality in Massachusetts and Connecticut in 1883, 1887, and 1888, though apparently not so bad as in 1889.

The chart (Fig. 2) shows the distribution of the various years as to the weather conditions already discussed; that is, the temperature in May and June, here expressed as the sum of the monthly mean temperatures, and the number of days with 0.01 in. or more rain in July and August during the

² Halsted, B. D. Some fungus diseases of the cranberry. Part II. The cranberry scald. New Jersey Agr. Exp. Sta. Bul. 64: 16-40. 1889.

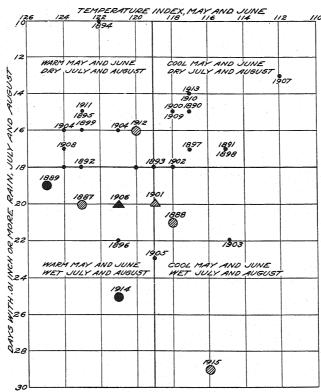


Fig. 2. Temperature during May and June and frequency of rainfall during July and August at Middleboro, Mass., 1887 to 1915, and relatively abundance of fruit rots in cranberries so far as records are available. Circular symbols same as in fig. 1; black triangle, very poor in New Jersey and no record for Massachusetts; triangle with cross-hatching, poor in New Jersey and no record for Massachusetts; small black dots, no record of unusual bad keeping qualities in either New Jersey or Massachusetts.

period 1889 to 1915, the period during which regular weather observations were made at Middleboro, Massachusetts. This weather observation station, while not located near a cranberry bog, as is that at East Wareham, was, during the period indicated, the nearest to the main cranberry-producing area. During this period 5 years, 1887, 1889, 1896, 1906, and 1914, fell within the group having weather believed to favor the increase of rot-producing fungi. Of these 5 years, 2, 1889 and 1914, are known to have had crops of very poor keeping quality, and 1, 1887, a crop of poor quality. The other years known to have produced crops of poor quality, 1888, 1912, and 1915, fell in the group having 1 unfavorable factor.

It would be of great interest if records could be obtained for the years 1896 and 1906, as well as for the 2 falling close to this group, namely, 1901

and 1905. Such records are not directly available, but what is known of the crops of those years in New Jersey furnishes indirect evidence that the losses from rot for the years 1901 and 1906 at least may have been larger than normal. While the cranberry crops of Massachusetts and New Jersey vary, as regards their keeping quality, to some extent independently of each other, it has been observed during the last 20 years that, when the crops of either State have been conspicuously poor in keeping quality, the crop of the other State has been below normal in this respect. It may then be worth while to note that the cranberry crop of New Jersey is known to have been of unusually poor keeping quality in 1901 and 1906.

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EFFECTS OF ANAEROBIC CONDITIONS ON THE GROWTH OF THE COTTON-ROOT-ROT FUNGUS, PHYMATOTRICHUM OMNIVORUM

D. C. NEAL AND R. E. WESTER (Accepted for publication March 21, 1932)

INTRODUCTION

The cotton root rot caused by the fungus *Phymatotrichum omnivorum* (Shear) Duggar has been studied extensively since 1888 and is of recognized importance in the agriculture of the Southwest. Although much study, by various workers, has been given this disease, there still are numerous questions relating particularly to the physiology of the fungus that have not been investigated. Among these is a more thorough knowledge of the factors necessary for optimum growth of the fungus in the soil. It has been claimed by some (8) that the heavy black-waxy soils of Texas, being poorly aerated, furnish favorable conditions for the growth of the fungus, and others have reported that deep tillage (1, 2, 3), clean fallows (5), or fallow treatments combined with subsoiling (4), in some cases, have greatly reduced the infestation in cotton fields. Deep-seated infestations of the fungus in tree roots buried below the plow sole also have been reported (6, 7).

Although it is generally recognized that most organisms, excepting certain anaerobic bacteria and fungi, can live for only a short time unless supplied with free oxygen, yet little is known regarding the behavior of normally aerobic fungi under anaerobic conditions.

In order to gain further information regarding these points, the writers have made comparisons between the growth of the root-rot fungus in normal atmosphere, in an atmosphere of nitrogen, and in the presence of varying amounts of carbon dioxide.

MATERIALS AND METHODS

An isolation of *Phymatotrichum omnivorum* obtained from San Antonio, Texas, was used in these experiments. After uniform growth was established on neutral carrot agar, transfers of equal sizes were made to 5 Petri dishes and placed in a desiccator in which normal atmosphere was replaced with nitrogen by the pyrogallic acid method; the desiccator, with the enclosed cultures of *P. omnivorum*, being sealed by means of petrolatum and incubated at 29° C. for 8 days, together with 5 checks.

Further studies were conducted by growing the fungus in 25, 50, 75, and 100 per cent carbon dioxide in an anaerobic culture jar, with 50 cc.

Erlenmeyer flasks instead of Petri dishes as the containers for the organism. This change was necessary in order to reduce the possibilities of contaminating the cultures. The carbon dioxide was generated by adding hydrochloric acid to marble. Then the gas was bubbled through water and caught in a graduated container. A layer of lubricating oil, ½-in. thick, was used in separating the water from the carbon dioxide in this container. After generating a container full of the gas it was forced into the anaerobic jar through rubber tubing by admitting water to the carbon dioxide container below the oil layer. As water was added, the oil layer would rise and force the carbon dioxide into the anerobic jar. This method permitted the measurement of the volume of gas used and the calculation of the approximate concentrations employed.

RESULTS

The results obtained in growing the fungus in normal atmosphere, in an atmosphere of nitrogen, and in varying concentrations of carbon dioxide are given in tables 1 and 2; and the effect of aerobic and anaerobic conditions upon growth after a period of 8 days is illustrated in figure 1.

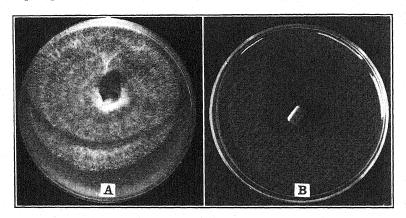


Fig. 1. Growth of *Phymatotrichum omnivorum* after 8 days on neutral carrot agar. A. In normal atmosphere. B. In an atmosphere of nitrogen.

TABLE 1.—Growth of Phymatotrichum omnivorum under aerobic and anaerobic conditions

	Diameter of colonies in millimeters ^a						
Atmosphere	Petri dish number						
	1	2	3	4	5	Ave.	
	mm.	mm.	mm.	mm.	mm.	mm.	
Nitrogen	0.0	0.0	0.0	0.0	0.0	0.0	
Check	80.0	81.0	80.0	83.0	82.0	81.1	

a Measurement recorded at the end of 8 days.

TABLE 2.—Effect of carbon dioxide on the growth of Phymatotrichum omnivorum

	Diameter of colonies in m					illimetersa		
Atmosphere	Amount of CO ₂		Flask numbers					
		1	2	3	4	5	Ave.	
	%	mm.	mm.	mm.	mm.	mm.	mm.	
Carbon dioxide	25.0	45.0	42.0	43.0	44.0	42.0	43.2	
Check		45.0	45.0	44.0	45.0	44.0	44.6	
Carbon dioxide	50.0	22.0	28.0	25.0	22.0	29.0	25.2	
Check		45.0	44.0	45.0	40.0	43.0	43.4	
Carbon dioxide	75.0	10.0	11.1	11.1	11.1	10.0	10.7	
Check		45.0	46.0	45.0	46.0	47.0	45.8	
Carbon dioxide	100.0	0.0	0.0	0.0	0.0	0.0	0.0	
Check		45.0	44.0	46.0	45.0	45.0	45.0	

a Measurements recorded at the end of 5 days.

DISCUSSION AND SUMMARY

The data reported show that growth of *Phymatotrichum omnivorum* is inhibited by anaerobic conditions and is notably restricted by concentrations of carbon dioxide greater than 25 per cent. Although the fungus does not grow in an atmosphere of 100 per cent nitrogen or carbon dioxide, it is not killed and, soon after being returned to aerobic conditions, growth occurs.

How long this organism is able to survive under anaerobic conditions is not known, but further experiments are being made.

The aerobic requirements for growth of the fungus, as indicated by these experiments, may explain the differences in root-rot infection previously referred to in this paper, which have been observed in cotton fields following such treatments as subsoiling, or fallowing, or fallow combined with deep tillage.

Subsoiling infested fields during the late summer or early fall may provide the necessary aeration for the germination of the sclerotia, thereby lessening the chances of winter survival of the fungus.

The recurrence of the disease in areas that have been kept in clean fallow for 3 and 4 years may be due to anaerobic conditions of the subsoil, especially in the highly calcareous, black-waxy lands of Texas, as well as to deep-seated infections of the fungus in tree roots below the plow sole. The sclerotia of the fungus, found frequently below the ordinary depth of cultivation in these soils, may remain dormant for long periods until sufficient

oxygen to stimulate their germination is provided either by the growth of plant roots, by subsoiling, or by deep natural cracking of the soil as occurs in dry seasons.

BUREAU OF PLANT INDUSTRY,

U. S. Department of Agriculture, Greenville, Texas.

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PHYTOPATHOLOGICAL NOTES

The Rôle of Diseases in Plant Quarantines.—A circular, issued periodically by the Bureau of Plant Quarantine of the United States Department of Agriculture, lists 42 Federal plant quarantines and orders, of which 22 are foreign and 20 domestic. Of the 22 foreign quarantines, which restrict or prohibit the importation of plants and plant products from foreign countries, 7 are maintained because of diseases alone, 9 because of insects alone, and 6 because of both insects and diseases.

Of the 20 domestic quarantines 4 are imposed because of diseases alone: Black Stem Rust (Quarantine No. 38); White Pine Blister Rust (No. 63); Woodgate Rust (No. 65); and Phony Peach (No. 67). Two domestic measures, the quarantine on Sugarcane (No. 16) and that on Narcissus Bulbs (No. 62) is based on both insects and diseases.

Plant diseases thus constitute an essential feature in 6 of the 20 domestic quarantines and in 13 of the 22 foreign quarantines; altogether, plant diseases are a fundamental part of 19 of the 42 Federal quarantines.

The 7 foreign plant quarantines, based on diseases alone, may be summarized as follows:

- Quarantine No. 3, the Potato Wart Quarantine, forbids the importation of potatoes from specified countries and localities known to have potato wart.
- No. 7, the White Pine Blister Rust Quarantine, established to prevent the entrance of this disease, forbids the importation of 5-leaf pines and *Ribes* and *Grossularia* spp. from parts of the world where the disease is known to occur.
- No. 19 prohibits the entrance of nursery stock, buds, and scions of citrus from the world at large because of citrus canker and other diseases.
- No. 24. By this quarantine the importation of corn and related plants in any stage (except corn as seed) from certain Asiatic areas, the Pacific islands, and Australasia is prohibited because of the Physoderma diseases and downy mildews (*Sclerospora* spp.). Shelled corn may enter from these countries under strict regulation.
- No. 28 aims to prevent the further entrance of citrus canker by excluding all citrus fruits except oranges of the mandarin class from the general Asiatic and Pacific island regions, and South Africa, excepting Australia, Tasmania, and New Zealand.
- No. 34. Seeds, plants, and cuttings of bamboo are prohibited entry from all parts of the world in order to keep out numerous diseases affecting bamboos, especially the bamboo smut, *Ustilago shiraiana*.
- No. 59, Flag Smut. All species and varieties of wheat, unless milled or processed, are forbidden entry from countries where flag smut is known to exist.

Foreign quarantines in which injurious diseases are coupled with insects as a basis for the measures taken are as follows:

- No. 15 aims to protect sugar-cane culture in the United States by excluding all living canes or living cuttings or parts of sugar-cane.
- No. 37 covers nursery stock, plants, and seeds from all countries and provides for the regulated entry of such of these as may be imported.
- No. 41. Entry of Indian corn and related plants from all foreign countries is made subject to regulations on account of the European corn borer and other dangerous insects and plant diseases.
- No. 55 is concerned with seed or paddy rice, the unhulled and unpolished grain used for seed. Such seed rice is not permitted entry, except under regulation from Mexico.
- Potato Regulations. A special set of regulations has been issued to govern the importation of potatoes from all countries from which they are not excluded by Quarantine No. 3.
- Plant Safeguard Regulations. These regulations are intended to provide means for the safe handling of restricted or prohibited plants and plant products brought into a port of entry for reexport to some other country or as ships' stores or other case of temporary stay. Such materials may be safeguarded against the escape of pests under these regulations while they remain in United States territory.

It is noted that in connection with all quarantines of a prohibitory nature special provision is made in the Plant Quarantine Act for importations by the Department of Agriculture, a feature that enables the Department to introduce under safe conditions desirable new varieties, rare plants, useful crop plants, or experimental materials.—W. A. McCubbin, Bureau of Plant Quarantine, Washington, D. C.

The Occurrence of the Perfect Stage of Phomopsis mali in the United States.—The importance of apple-foliage diseases of the frog-eye type in the fruit-growing sections of northwest Arkansas was stressed by Scott and Rorer¹ in 1907. These investigators found that the leaf-spot diseases were responsible for premature defoliation, small fruit of poor quality, weakened trees, immature fruit buds, and the reduction of the crop in succeeding years. While the general recognition by the orchardists that a definite spray program must be followed every year for the control of apple diseases has doubtless reduced the importance of apple-foliage diseases in recent years, the fact remains that in many orchards apple-leaf spots still are present every year. In some years they become so numerous that they produce injuries almost as severe as those observed by Scott and Rorer.

During the growing season of 1931 the writer repeatedly secured cultures of a species of Phomopsis from apple-leaf spots from orchards in the

¹ Scott, W. M., and J. B. Rorer. Apple leaf spot caused by Sphaeropsis malorum. U. S. Dept. Agr., Bur. Plant Indus. Bul. 121: 47-54. 1907.

vicinity of Fayetteville, Arkansas. A comparison of these cultures with type material has demonstrated that they are identical with *Phomopsis mali* Roberts.

On November 6, 1931, one of these cultures, which had been transferred to sterile apple twigs in test-tubes on August 8, was found to have several long beaks or neck-like structures projecting above the surface of a twig. These structures were found to be the beaks of perithecia that had formed beneath groups of pycnidia. Following the discovery of these perithecia other cultures of *Phomopsis mali* were transferred to sterile apple twigs and perithecia eventually formed in 10 different isolations out of a total of 34 tested.

A study of the morphology of the perithecia, asci, and spores indicated that the fungus was a member of the genus Diaporthe.

Single ascospore isolations were made from various perithecia and transferred to potato-dextrose agar. Pycnidia formed on the agar and conidia began to ooze from them in 28 days. An examination of the pycnidia and the spores showed them to be identical with those of *Phomopsis mali*. After 90 days a new generation of perithecia, with typical Diaporthe characters, appeared in these cultures. Similar results were secured when ascospores were transferred to sterile apple twigs in test-tubes, but the rate of development was accelerated, conidia appearing in 19 days and perithecia in 54 days. The cultures from ascospores gave rise to *P. mali* conidia that, when transferred to sterile apple twigs, produced cultures in which perithecia appeared after 76 days.

It is evident, as the result of these experiments, that a genetic connection exists between *Phomopsis mali* and the Diaporthe and, conversely, that the Diaporthe is the perfect stage of *P. mali*.

In the apple-twig cultures the globose to globose-flattened perithecia are embedded in a matrix of fungus hyphae, pycnidia, and twig tissues. The perithecia occur singly or in groups but are always separately erumpent by curved, twisted, hairy, black (except at the extreme tip) beaks 1 to 4 mm. long.

The outer wall of the perithecium is black and membranous, enclosing an inner layer of brown tissue that surrounds the asci. The asci are approximately 40 to 60 by 5 to 7 μ , containing eight 2-cell, hyaline spores measuring 9.5 to 13.5 by 2.7 to 3.8 μ . The ascospores are obtuse at both ends, slightly constricted at the septum, and each cell contains 2 oil drops. No paraphyses were observed.

Kidd and Beaumont,² after a comparison of American and European cultures of Phomopsis, decided that *Phomopsis mali* is probably the imper-

² Kidd, M. N., and A. Beaumont. Apple rot fungi in storage. Trans. Brit. Mycol. Soc. 10: 98-118. 1924.

fect stage of Diaporthe perniciosa Marchal. The morphological characters of the ascomycete that developed in the writer's cultures of P. mali agree very well with the published descriptions of D. perniciosa, and the fungus may be referred to this species pending a critical study of European cultures and exsiccati.—John C. Dunegan, Division of Horticultural Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, cooperating with the Department of Plant Pathology, University of Arkansas.

Another Gall-Forming Bacterium.—In the spring and summer of 1932, a soft nodular-type gall was found destroying grafted plants of Gypsophila paniculata Linn. (baby's breath) in a New Jersey nursery. The galls occurred at the crown and root in the region of the graft; none was found on the aerial stems. In general appearance most of the galls were not so globular as those of the typical crown ball produced by Bacterium tumefaciens Sm. and Town. but usually produced a flat nodular growth similar to the outgrowths of the pocket disease of sugar beets produced by Bact. beticola (Sm., Br., Town.) Poteb.

The Gypsophila galls ranged from $\frac{1}{2}$ cm. to 3 cm. in diameter (Fig. 1), but there was evidence of gall decay in some plants, which indicated the

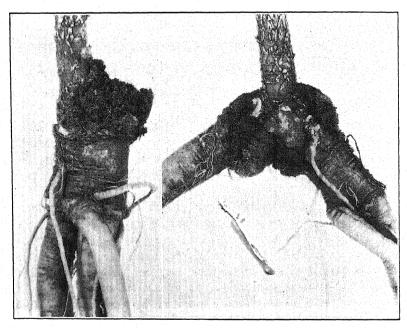


Fig. 1. Galls on Gypsophila (baby's breath) from a nursery in New Jersey. $\times 1$. Photographed on May 25, 1932.

existence of still larger galls. When cut across, the tumors were sound, creamy white, with water-soaked areas, a condition unlike crown gall, olive gall, or pocket disease of sugar beets. The two latter types have pocket-like areas in mature tumors filled with a gummy substance or from which a slimy ooze exudes, depending on the age and condition of the gall. Crowngall tissue on most hosts, when sound, is white and without water-soaked areas.

The organism isolated from the Gypsophila gall is a yellow one and its infectious nature was proved by inoculations into the crown and root of Gypsophila plants. In less than 2 weeks after inoculation definite outgrowths developed at the crown and in 2 months the tumors were $2\frac{1}{2}$ cm. across. Inoculations made into the aerial stems of Gypsophila plants produced cankers instead of galls. Three different sets of inoculations made into sugar beets of different ages failed to produce infection, as did inoculations into several other plants, such as Ricinus, Bryophyllum, tomato, and garden balsam.

The organism can grow at very low and high temperatures for a plant pathogen; it is a motile rod whose flagella have been stained and found to be bipolar, and it has been recovered from the galls produced by inoculations.

So far as the study has progressed, the organism appears to be another gall-forming bacterium, but considerable comparative cultural and morphological work needs to be done to be sure it is not related to the yellow, tumorforming sugar-beet organism, *Bacterium beticola*.—Nellie A. Brown, Division of Horticultural Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture.

A Convenient Method of Bleaching and Clearing Leaves.—In the course of a program of histological studies of the genus Ribes L., it was found necessary to bleach and clear a large number of leaves preparatory to determining the character, frequency, and distribution of stomata, trichomes, hydathodes, and crystal druses. A commercial grade of sodium hypochlorite, with or without the addition of sodium hydroxide (flaked lye), has proved more convenient and satisfactory than any of the reagents commonly used for this purpose.

In brief, the procedure followed consisted in processing leaf tissues in air-tight containers, such as corked shell vials or small screw-top glass jars, containing an aqueous solution of commercial sodium hypochlorite and lye, until sufficiently bleached and cleared for microscopic examination.

The most satisfactory concentrations of the two substances in the solution range from 5 to 20 per cent by volume of sodium hypochlorite and from 1 to 5 per cent by weight of sodium hydroxide, depending upon the

type of material under investigation. A solution of 10 per cent commercial sodium hypochlorite and $2\frac{1}{2}$ per cent sodium hydroxide gave satisfactory results in many cases, but a solution containing 20 per cent of the hypochlorite and 5 per cent sodium hydroxide proved more efficient. The latter solution was the one most frequently used and was sufficiently strong to bleach and clear all leaves that were worked upon. A solution of 10 per cent hypochlorite and 5 per cent sodium hydroxide, however, is recommended for preliminary work with unfamiliar material. The presence of an appreciable quantity of sodium hydroxide hastens the process, results in clearer materials and increases the ease with which the leaves can be "wetted" in the solution.

The time required for clearing the leaves varies from 12 to 96 hours, depending upon the type, size, and condition of the leaves and the strength of the solutions used. The solution seems to penetrate the leaf through cut edges, or other breaks in the epidermal layers, and not directly through uninjured tissues. Consequently, small pieces of leaf tissue require less time to be processed than whole leaves. In some cases, one or more changes of the processing solution may be necessary.

While the leaves are in the clearing and bleaching solution, some maceration may be expected. It may therefore be necessary to pour them into a large pan of water and to straighten them out upon a submerged piece of glass before undertaking microscopic examination. Whole leaves or large pieces of leaf tissues are conveniently manipulated for observation on the cover glasses of lantern slides.

Commercial sodium hypochlorite appears to keep indefinitely in the bottle in which it is sold as long as the bottle remains tightly stoppered. Diluted solutions do not seem to keep particularly well, but, when made, should be stored in dark, tightly-stoppered bottles in a cool place.

The process is equally satisfactory for fresh material, dried (herbarium) material and for material preserved in formalin-acetic alcohol, but cannot be used for material to be imbedded in paraffin, because the cell contents are dissolved by the solution and all details are lost. This process has been used on the leaves of a variety of California plants including several Ribes species, which, as a rule, are quite tanniniferous; and upon Arctostaphylos patula Greene, green manzanita; Myrica californica Cham., wax myrtle; and Castanopsis sempervirens Dudley, bush chinquapin, all of which bear what might be termed durable leaves.

The method given herein is, of course, intended for temporary mounts. In all microscopic work on the leaves bleached and cleared in the manner described, weak illumination has been found to be more satisfactory than strong illumination.—Clarence R. Quick and Frank A. Patty, Division of Blister Rust Control, Bureau of Plant Industry, United States Department of Agriculture.

The Nonspecificity of the Brown-Ring Symptoms in Narcissus Attacked by Nematodes.—The purpose of this note is to call attention to the fact that the brown-ring symptoms of narcissus bulbs, if of true or apparent nemic origin, are not specific for Tylenchus dipsaci Kühn. According to our observations, various other species, such as Aphelenchoides fragariae Ritzema Bos, A. parietinus (Bastian), Aphelenchus avenae Bastian, and even Cephalobus striatus deMan, may produce or be connected with brown rings. Development and character of the symptoms are in all instances very similar (Fig. 1, A-D and legend), although none of the forms mentioned

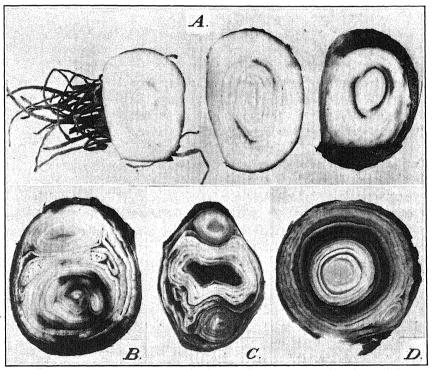


Fig. 1. A. Rather early stage of brown-ring symptoms produced by Tylenchus dipsaci in a narcissus bulb. The crosscuts from right to left show how the disease proceeds from the neck region of the bulb downwards. B. Brown rings produced by Aphelenchoides parietinus. Aphelenchus avenae produces very similar rings. C. Brown center in the neck region of a narcissus bulb containing large numbers of Cephalobus striatus. D. Brown rings produced by Aphelenchoides fragariae.

seems to be so virulent and of such high pathogenicity as T. dipsaci. In narcissus bulbs affected by any of these nemic forms the disease normally

¹ Synonyms of this form are: Aphelenchoides subtenuis (Cobb), A. olesistus Ritzema Bos, and A. ritzema-bosi Schwartz.

begins at the neck, the ring symptoms proceeding downwards. Cephalobus striatus was observed in two cases, in both of which only the center portion of the neck was affected, as shown in figure 1, C. It is possible that this species, which, according to our knowledge, acts more as a saprophyte, simply takes advantage of the weakened condition of the attacked region as sometimes produced by faulty hot-water treatment. With the exception of T. dipsaci, Aphelenchoides fragariae is most frequently found, of the forms mentioned.—G. Steiner and Edna M. Buhrer, Division of Nematology, Bureau of Plant Industry, U. S. Department of Agriculture.

Diaporthe citri (Faw.) Wolf, the Perfect Stage of Phomopsis citri and P. californica.—Study of perfect stages and of numerous isolations of these previously supposed different species^{1,2} has convinced the writer that they should now be considered as one species. The perfect stage of Phomonsis californica Faw, has been studied and no characteristics have been found that would distinguish it from the Diaporthe stage described by Wolf* for P. citri Faw. Moreover, the writer had the opportunity to examine a large number of isolations made by R. G. Tomkins, of Cambridge, from fruits from various parts of the world: Palestine, South Africa, Brazil, Spain, Florida, etc., and no characteristic differences that would seem to separate these isolations from those of California could be detected. The writer has also examined certain isolations sent by K. Nakata, of Japan, which appear to be the same fungus. It is, therefore, believed that this species is of world-wide distribution on Citrus. Melanose, the effect so prevalent in Florida and certain other countries, is believed to be largely dependent on climatic conditions, especially a proper combination of rainfall, temperature, and growth conditions. Although the fungus is present in climates like California and Palestine, producing stem-end rot, it is rarely evident as melanose. Melanose has been found occasionally in a mild form in California.—H. S. FAWCETT, Riverside, California.

¹ Fawcett, H. S. The cause of stem-end rot of citrus fruits (*Phomopsis citri* n. sp.). Phytopathology 2: 109-113. 1912.

² Fawcett, H. S. A new Phomopsis of Citrus in California. Phytopathology 12: 419-424. 1922.

³ Fawcett, H. S., and C. L. Shear. Discovery of the perfect stage of a Phomopsis on lemon bark. (Abst.) Phytopathology 19: 1138. 1929.

⁴ Wolf, F. A. The perfect stage of the fungus which causes melanose of Citrus. Jour. Agr. Res. 33: 621-625, 1926.

BOOK REVIEW

Barton-Wright, E. C. Recent Advances in Botany. 287 pp. 60 figs. P. Blakiston's Son & Co., Philadelphia. 1932. Price \$4.00.

This volume, which aims to present only a certain few of the recent advances in botany, makes no reference to work in general cytology or genetics; the 9 chapters concern general morphology (1), paleobotany (1), taxonomy (1), mycology (3), algae (2), and virous diseases of plants (1).

In the chapter on general morphology special attention is given to the relation between size and form, the phyllode theory of the monocot leaf, and carpel polymorphism. The chapter on paleobotany deals especially with the carboniferous bryophytes of the genus Hepaticites Walton and the Caytoniales of the Mesozoic; this latter group opens up an entire new chapter in the history of the Angiosperms. An excellent thread to guide the reader through the maze of modern taxonomic literature is given in the chapter on the species problem, with emphasis on the conflicting claims of the habitat (ecospecies) and of the genetical composition (genospecies) in determining "species," with especial stress on the work of Turesson.

Chapter 4 reviews papers on the life histories of the various groups of fungi, with emphasis on the work of Cook and Schwarz on Plasmodiophora and of Wilson on *Reticularia lycoperdon*. Attention is also given the life histories of the Chytridiales, Kanouse's work on the classification of the Oomycetes, and some of the papers on the double nuclear fusion in the Ascomycetes. In regard to this last problem, the recent work seems to support the view that there are two nuclear fusions in the life cycle and that reduction is accomplished in two stages, contrary to the opinion of Claussen.

The remaining two chapters on the fungi are concerned with the problems of heterothallism and mycorrhiza. In addition to the original work of Blakeslee on the Phycomycetes, similar conditions have been reported by many workers in the Ascomycetes and Basidiomycetes. In this last group, the problem becomes especially complex because of the polyspory present in many forms. Most of the work has dealt with facts rather than with explanations, although Gwynne-Vaughan has attempted to explain heterothallism in the Ascomycetes and Hymenomycetes on the basis of nutrition. Assuming that certain strains (–) can extract certain food substances essential for fruiting body formation from the substrate, while other strains (+) can extract others equally essential, then the fruiting bodies would be formed only when the two strains met. If in "quadrisexual" forms, such as Aleurodiscus polygonius and Coprinus lagopus, the 4 characters are segregated in Mendelian fashion, the same explanation would hold.

The chapter on mycorrhiza is concerned chiefly with the work on the orchids, heaths, Bryophytes, and forest trees. The precise rôle of the endophytic fungi still remains to be cleared up, although the evidence points more and more in favor of the view that the fungi are of genuine benefit to the "host," either to hydrolyze carbohydrates (orchids), increase the N supply (heaths), or aid in getting mineral salts (trees). The double infection theory of Peyronel is discussed, according to which it is assumed that the infection is of a composite nature, an initial infection paving the way for a second (and different) form.

The two chapters on the algae are devoted to the Phaeophyceae and the Florideae, with emphasis upon their cytology and life histories in an attempt to straighten out the more or less confused taxonomic questions in these groups and to clarify the gametophyte-sporophyte relationships.

The last chapter (virous diseases) is doubtless of most interest to the readers of this journal and for this reason will be discussed in somewhat greater detail. Although these diseases have been known since 1892, only in the last decade has their study gained much headway. Since they are present in herbaceous more than in woody plants, the former group has received the most serious study (notably the Solanaceae). The disease is generally accompanied by chlorosis of the leaves and necrosis of the phloem. Although death seldom results, recovery occurs very rarely if ever. Storey has claimed the recovery of sugar-cane from the streak disease and Robbins of sugar beets from mosaic. In many of these cases, however, the symptoms have probably only been masked.

The viruses are, in general, very resistant to high temperatures and to most of the common antiseptics (acids, alkalis, alcohol, etc.). Formaldehyde was the most efficient in destroying the virus of tobacco mosaic, which withstands heating for 2 days at 80° C., simple storage for 5 years, and 90 per cent alcohol acting for 1 hr. Cucumber-mosaic virus is not so resistant; it loses its infecting power after heating up to 70°, after standing for 7 days, or when treated with 40 per cent alcohol. Other viruses range, for the most part, between these two.

No satisfactory system of classifying or naming these diseases has yet been developed. As many as 8 different viroses have been described for tobacco (Johnson) and 5 to 7 for the potato. Work is now being done on keys to distinguish the various kinds, which are thus far distinguished by different letters of the alphabet. Some workers have even anticipated the discovery of future similar virous diseases in specific hosts and have named a disease as "virus A" when there is as yet no "B." This the author considers to be the source of needless confusion.

The best work on the subject has been done on the question of transmission. Tobacco mosaic is spread very readily even by rubbing a diseased

plant with the fingers and then a healthy plant. Grafting invariably results in transmission, while transmission through the seed is comparatively rare (eucumber mosaic). In nature the chief agents are insects, for which the reader is referred to the summary by Kenneth Smith (Biol. Rev. 6). In potato leaf roll, aphids (Myzus persicae) are the chief carriers. In some cases one insect may transmit several diseases or one disease may be transmitted by several insects, while in other cases there is great specificity between disease and vector. In many cases, however, the transmission is not a simple mechanical transfer; a certain incubation time must elapse between feeding and transmission, which points to a biological rather than chemical nature of the disease.

Smith found in experimenting with tobacco and potato mosaic that there was a difference between the types of infection produced by needle inoculations and aphid transfers. In the latter case, there was little increase in virulence with successive inoculations such as occurred with the needle transfers. The aphid thus seems to fail to transmit some element of the virus that produces the more lethal and necrotic symptoms. The virus is thus divided into x and y elements. Work by Allard and others has shown that it is possible for a plant to be normal in appearance and still be a "carrier" of a virous disease.

The causative organism has been sought at various times in bacteria, protozoa, and mycetozoa, all of which were found in plants infected with virous diseases. Of late, attention has been centered on the X-bodies (Goldstein) associated with the protoplasm and generally near the nuclei in infected plants. These X-bodies are granular, deeply staining, vacuolate, and roughly spherical. Since they are more or less amoeboid, are proteinaceous, have been associated by some workers with mitochondria, and seem to increase and divide, they have been thought to be living entities, but this question remains to be settled. The work of Henderson Smith on the X-bodies in Solanum nodiflorum tends to show that, although of a protein nature, they are not organisms. At any rate, no plant virus has ever been made to multiply outside its host.

As to the physiology of the infected plants, comparatively little has been done. During the disease, carbohydrates accumulate in the leaf accompanied by the necrosis of the phloem. That the latter is not entirely the cause of the former, however, is shown by the fact that the accumulation of starch in leaves (potato roll) may occur before the necrosis of the phloem (Murphy). Translocation is the most difficult problem to explain in leaf-roll plants. Although no sucrose is found in the petioles, some sugars get down to the tubers, and it is concluded that there must be a slow leak of hexoses via the ground parenchyma of the petioles and stems. In the spike disease of

Santalum, the pH value is lowered in diseased leaves, which were also found (Iyengar) to be deficient in K and Ca. Diastase activity, on the other hand, is greater in diseased than in healthy leaves.

In most cases the virus seems to move in the plant in the phloem, as evidenced by ringing and similar experiments, but Caldwell claims that under exceptional conditions aucuba virus in tomatoes may be made to travel in the xylem but is incapable of leaving it to infect the living tissues. Only when the leaves were crushed and the virus could escape from the conducting vessels was infection possible. Rate of movement of "curlytop" virus in the sugar beet was found by Severin to be 10 cm. per hour, while Storey found the virus of corn mosaic to travel at 10 to 20 cm. per hour.

This little volume will be found an extremely valuable addition to the library of the nonspecialist who wishes to keep pace with the progress in related fields. In a work of this nature, the materials must be selected with great care and presented critically to be of the greatest value. In this regard the author has done a good piece of work. Furthermore, each chapter is prefaced with sufficient introductory, historical matter to make the following discussion intelligible. In spite of such minor slips as on page 273, where pH value and H-ion concentration are considered synonymous, the book is well written and quite up to the high standard of the "Recent Advances" series of which it is a part.—Oran Raber, Biological Abstracts, Washington, D. C.

THE PRINCIPLES OF PLANT QUARANTINE

INTRODUCTION

For many years a definite need has existed for a simple but adequate statement setting forth the principles that ought to be followed in the establishment and enforcement of plant quarantines. If such a statement could be prepared so as to embody the best opinion available on the subject it would serve as a valuable guide in practice in a field that has developed ever-increasing complexity. In 1926 the National Plant Board undertook the preparation of such a statement. In succeeding years the original draft has been subjected to critical revision, both by the National Plant Board itself and by members of the 4 regional plant boards. From this process of revision the statement of quarantine principles may be said to have emerged with the approval of the quarantine and regulatory representatives of all 48 States. As thus finally approved it was issued July 25, 1931, by the National Plant Board, under the title, "Principles of Plant Quarantine." As a valuable contribution in the regulatory field it is reproduced for the information of plant pathologists, who logically must share with entomologists a national responsibility for the wise future development of plant quarantines.

PRINCIPLES

- I. Definition. A quarantine is a restriction, imposed by duly constituted authorities, whereby the production, movement or existence of plants, plant products, animals, animal products, or any other article or material, or the normal activity of persons, is brought under regulation, in order that the introduction or spread of a pest may be prevented or limited, or in order that a pest already introduced may be controlled or eradicated, thereby reducing or avoiding losses that would otherwise occur through damage done by the pest or through a continuing cost of control measures.
- 2. Basis in Logic. Since the ends to be attained by a quarantine and the measures required by it could not be undertaken by private individuals or groups, involving as they do restrictions on areas, persons, or activities for the benefit of wider interests or the public at large, resort to regulation imposed by public authority is logical.
- 3. Necessity. Establishment of a quarantine should rest on fundamental prerequisites, as follows: (1) the pest concerned must be of such nature as to offer actual or expected threat to substantial interests; (2) the proposed quarantine must represent a necessary or desirable measure for which no other substitute, involving less interference with normal activities, is available; (3) the objective of the quarantine, either for preventing introduction or for limiting spread, must be reasonable of expectation; (4) the economic gains expected must outweigh the cost of administration and the interference with normal activities.

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- 4. Legal Sanction. A quarantine must derive from adequate law and authority and must operate within the provisions of such law.
- 5. Validity. A quarantine established for the purpose of attaining an objective other than that which it indicates or defines is open to serious criticism, even though the actual objective is itself desirable.
- 6. Public Notice. If the circumstances will permit, public notice of a proposed quarantine should be given and those interested should be invited to contribute facts in their possession. But if the objective would be defeated by the delay required for such notice and discussion, duly-constituted authorities should assume responsibility for the decision to impose or withhold quarantine action.
- 7. Scope. The extent of restrictions imposed by a quarantine should be only such as are believed necessary to accomplish the desired end, but on the other hand the objective of a quarantine should not be jeopardized by omission of any necessary restriction.
- 8. Relation to Eradication. If a quarantine is imposed in order that eradication of a pest from a given area may be undertaken, the restrictions involved may properly be relatively extensive, because of the importance of the objective sought, and because the time through which the quarantine will operate may be expected to be relatively limited.
- 9. Relation to Retarding Spread. If a quarantine is imposed for the purpose of limiting or retarding spread of a pest, but without expectation of eradication, the restrictions imposed should be such as are in line with the objective of the quarantine and should recognize the fact that continuance of the pest in the area where it is established, or possibly its spread in time to new areas, is accepted.
- tions between public authorities. Since quarantines usually involve relations between public authorities, such as those of the government of one country with that of another, or of Federal and state governments, or of state government and local authorities, the cooperative relationship that is necessary to adequate enforcement should be clearly recognized and duly provided for.
- II. Cooperation of the Public. Because of the fact that the success of a quarantine requires that its restrictions be fully maintained, it is essential that all persons who are affected by it adhere to its requirements. In order that this end may be attained the administration of a quarantine should seek the intelligent cooperation of the public affected, rather than depend exclusively on police powers, the imposition of penalties, or resort to court action.
- 12. Clarity. In order that a quarantine may be administered readily and consistently, it should be designed with care, should be phrased clearly, and should be made as simple as is consistent with legal requirements and the objective to be attained.
- 13. Information Service. Since the persons affected by a quarantine may not reasonably be expected to possess full or accurate knowledge of the circumstances that make it necessary, or the nature and importance of the

aim sought, and since compliance with quarantine restrictions will be more complete if the objective and plans are understood, measures should be taken to set forth the conditions existing, the means to be employed, and the end to be attained, and these measures should be continued from time to time as the undertaking proceeds toward accomplishment.

- 14. Research. If an emergency requires the establishment of a quarantine before satisfactory biological data are available, provision should be made as soon as possible for extending the fund of biological knowledge. The authority that exercises the right to establish a quarantine should command or secure the means for biological research, both in order that the quarantine may be made more efficient, and in order that the restrictions may be lessened where possible. The need for resarch, however, should not be permitted to delay the establishment of a quarantine believed by authorities to be desirable, thereby jeopardizing the objective that might otherwise have been attained.
- 15. Modifications. As conditions change, or as further facts become available, a quarantine should promptly be modified, either by inclusion of restrictions necessary to its success or by removal of requirements found not to be necessary. The obligation to modify a quarantine as conditions develop is a continuing obligation and should have continuing attention.
- 16. Repeal. If a quarantine has attained its objective, or if the progress of events has clearly proved that the desired end is not possible of attainment by the restrictions adopted, the measure should be promptly reconsidered, either with a view to repeal or with intent of substituting other measures.
- 17. Notices to Parties at Interest. Upon establishment of a quarantine, and upon institution of modifications or repeal, notices should be sent to the principal parties at interest, especially to Federal and state authorities and to organizations representing the public involved in the restrictive measures.

NATIONAL PLANT BOARD, OFFICE OF THE CHAIRMAN, W. C. O'KANE, DURHAM, NEW HAMPSHIRE, JULY 25, 1931. The following addresses were omitted from the List of Members of The American Phytopathological Society, Supplement to Phytopathology, Vol. 22, No. 9, Sept., 1932.

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PATHOGENICITY AND CULTURAL CHARACTERS OF THE APPLE SOOTY-BLOTCH FUNGUS¹

R. C. BAINES AND M. W. GARDNER² (Accepted for publication March 21, 1932)

Cultures of sooty blotch have been obtained from the fruit of apple and wild crab apple and from the young twigs of 23 other species of trees, shrubs, and vines. In culture the fungus grows slowly and produces a tough, leathery, black, heaped-up thallus from which gelatinous spore masses ooze out in great abundance. With spores from pure cultures from apple and 14 other hosts, successful inoculation of apples was obtained. The incubation period in a cool moist chamber is 2 to 3 weeks and in the orchard, 1 to 2 months. On 12 of the wild hosts sporulating pycnidia were found in late May and early June. The spores usually are bicellular.

ISOLATION OF SOOTY-BLOTCH FUNGUS FROM VARIOUS HOSTS

According to Duggar (2, p. 367) Floyd found sooty blotch on 25 species of nonpomaceous trees and shrubs in the vicinity of Columbia, Missouri. Colby (1) observed sooty blotch on apple twigs and fruit, peach twigs, blackberry canes, and on the stems of black mustard. Doyle Zaring, working in this laboratory, obtained the sooty-blotch fungus from apple in pure culture in 1928 by means of tissue plantings in agar plates.

Pure cultures of what appear to be the same fungus have been obtained from sooty blotch (3) on the fruit of apple, *Pyrus malus*, and wild crab apple, *Pyrus coronaria*, and on the young twigs of the following 23 different hosts collected on various dates in the vicinity of La Fayette: hawthorn, *Crataegus mollis;* wild blackberry, *Rubus allegheniensis;* papaw, *Asimina triloba;* spice bush, *Benzoin aestivale;* sassafras, *Sassafras variifolium;* leatherwood, *Dirca palustris;* red elm, *Ulmus fulva;* white ash,

1 Contribution from the Botany Department, Purdue University Agricultural Experiment Station, La Fayette, Ind. Portions of a thesis submitted by Mr. Baines to the Graduate School of Purdue University in partial fulfillment of the requirements for the M.S. degree are incorporated.

² The writers wish to acknowledge the cooperation of Prof. C. L. Burkholder and the Department of Horticulture, the assistance rendered by Prof. E. J. Kohl, R. W. Samson, and L. B. Lockwood, and helpful advice received from Dr. C. L. Shear.

3 M.S. thesis, Purdue University, 1929. Unpublished.

Fraxinus americana; wild grape, Vitis cordifolia; hard maple, Acer saccharum; bladder nut, Staphylea trifolia; smilax, Smilax hispida; sycamore, Platanus occidentalis; willow, Salix nigra; prickly ash, Zanthoxylum americanum; dogwood, Cornus rugosa, and Cornus alternifolia; tulip tree, Liriodendron tulipifera; wahoo, Evonymus obovatus; bittersweet, Celastris scandens; red bud, Cercis canadensis; Kentucky coffee tree, Gymnocladus dioica; and white oak, Quercus alba.

From all of these hosts, except red elm, the fungus was isolated from tissue plantings. Small pieces of the host tissue bearing the fungus were first placed in 95 per cent alcohol to remove any air bubbles from the surface, then sterilized in mercuric chloride for 5 to 10 seconds, rinsed in water, and planted on poured plates of potato-dextrose agar. Since sooty blotch is superficial, the success of the method consists in sterilizing long enough to kill all of the contaminating organisms present, and presumably not long enough to kill all of the hyphae of the sooty blotch. The mycelium at the base of the aggregate masses of the sooty-blotch thallus probably escapes the sterilization by being in very close contact with the cuticle and protected from the disinfectant by the mycelium covering it.

The sooty-blotch fungus first became visible as a dark, compact mass of hyphae about the edge or on the surface of the tissue fragment in about 5 to 8 days at 25° C. The fungus grows slowly but extrudes gelatinous masses of spores promptly and in abundance. Because of other fungi growing from the tissue fragments, the fungus was not easily isolated by this method and was more easily isolated from apple than from the other hosts. In one instance, for example, it was isolated from 18 per cent of the 174 tissue plantings from apple fruits. The method described by Simmonds (7), in which the fragments of host tissue are not surface sterilized but are thoroughly washed in sterile distilled water, did not give satisfactory results. Groves (4), however, has made successful use of a modification of this method.

Very satisfactory results in isolating the fungus were obtained in the spring of 1931 by the dilution plate method, the inoculum being a suspension of the spores produced in the pycnidia on the twigs of the various hosts. By this method pure cultures were readily obtained from blackberry, bladder nut, hawthorn, leatherwood, smilax, willow, prickly ash, papaw, red elm, sassafras, and hard maple and were identical with the cultures previously isolated.

PYCNIDIA AND SPORE PRODUCTION ON THE VARIOUS HOSTS

Observations made in 1930 and 1931 showed that immature pycnidia were present on the twigs of the various hosts in the fall and that in the spring immature spores were present in these pycnidia. The course of de-

velopment of the pycnidia and spores in the spring of 1931 was followed by a microscopic examination of specimens collected at 2-week intervals from blackberry, white ash, white oak, papaw, smilax, bladder nut, red elm, hard maple, sassafras, and leatherwood. From March to June the pycnidia were found in progressive stages of maturity, and on May 30 mature spores were found in abundance. On June 6, after a heavy rain on the preceding day, it was found that nearly all of the pycnidia were empty. This would indicate that liberation and dissemination of the spores occurred during the rain on June 5.

Between March 31 and April 19, 1931, twigs of blackberry, white ash, papaw, smilax, bladder nut, red elm, hard maple, sassafras, leatherwood, willow, bittersweet, hawthorn, and prickly ash, bearing sooty blotch, were brought into the laboratory and placed in moist chambers at room temperature. The moist chambers were 2-quart Mason jars, containing about an inch of water and closed with loose cotton plugs. Under these conditions the maturity of the pycnidia was hastened and mature spores were obtained after an incubation period of 8 to 24 days, some as early as April 12.

From the spores produced in the pyenidia on these specimens incubated in the moist chambers plates were poured and pure cultures of the fungus obtained from all of the host species except white ash, sassafras, hard maple, and bittersweet. Pure cultures also were obtained from spores in the pyenidia on sassafras and hard maple collected on June 3. As has been previously mentioned, these cultures proved identical with those isolated from the tissue plantings.

Observations made in the early spring of 1932 indicate that sooty-blotch pycnidia were much more abundant on the various hosts than in the preceding season. This is not surprising because pycnidia are produced on mycelium that developed during the preceding year, and, as will be shown later, 1931 was more favorable for sooty blotch than 1930.

PATHOGENICITY OF THE CULTURES ON GREEN APPLES IN A MOIST CHAMBER

Before the cultural characteristics of the fungus were studied in detail, the pathogenicity of the cultures on apple fruits was first tested. In order to make inoculations on apples under controlled conditions and to maintain the fruits in sound condition for a period long enough for the fungus to develop, use was made of a large cool moist chamber in the greenhouse. This was a frame structure covered with burlap and kept wet with running water from a reservoir on top of the chamber. Evaporation from the wet burlap kept the temperature down to 18 to 22° C. in the chamber.

The fruits were washed and placed on galvanized-iron wire screen stretched over porcelain cake pans full of water, so that the pedicels of the apples extended down through the screen into the water. These pans were placed on shelves in the moist chamber. By this method freshly picked green apples were successfully kept over 2 months.

The apples were inoculated by atomizing them with a spore suspension from the cultures and subsequently were atomized with distilled water twice daily. With pathogenic cultures abundant infection of a severe type was apparent on the apples in 2 to 3 weeks. The noninoculated apples used as controls remained free from infection, as well as those inoculated with spores from nonpathogenic cultures. No differences in the type of infection with the various cultures were observed. The results of the in-

TABLE 1.—Inoculation of apples in a moist chamber with spores from sooty-blotch cultures from various hosts, 1930 and 1931a

Source of culture	Variety of apple	Number in- oculated	Percentage infected	Number of re- isolations
Apple	Walbridge	69	100	29
	Grimes	30	100	
((в	Ben Davis	20	100	
White ash	Walbridge	42	100	17
	Grimes	30	100	
	Ben Davis	10	100	
Papawb	Walbridge	47	100	6
	Grimes	29	100	3
	Ben Davis	10	100	
Hawthorne	Walbridge	24	100	8
	Ben Davis	20	100	
Sycamore	Grimes	12	100	14
	Ben Davis	10	100	
Grape	Walbridge	12	100	
"	Ben Davis	10	100	
Prickly ash	Grimes	12	100	13
Spice bush	Walbridge	12	100	16
Bladder nut	Ben Davis	10	100	1
Leatherwoodb		20	100	1
Red elm		10	100	2
Smilaxb		20	100	1
Willowb		20	100	
Mapleb		20	100	4
Sassafras		10	100	1
Controls	Walbridge	62	0	
	Grimes	24	0	
	Ben Davis	20	0	

a Inoculated between June 26 and October 18.

b Two cultures used.

c Three cultures used.

oculation of Walbridge, Grimes and Ben Davis varieties of apples in the greenhouse in 1930 and 1931 are summarized in table 1.

Sooty blotch was produced on apples in the greenhouse moist chamber by inoculations with spores from cultures from sooty blotch on apple (2 cultures), papaw, prickly ash, hawthorn (3 cultures), white ash, sycamore, spice bush, grape, smilax (2 cultures), willow (2 cultures), sassafras, red elm, hard maple (2 cultures), leatherwood (2 cultures), and bladder nut. No sooty blotch was produced by inoculating apples with spores from 2 cultures from blackberry.

In the successful inoculation of apples with spores from cultures, numerous dense black colonies were usually obtained. The blotch, representing the thallus of the fungus, apparently did not increase in size after becoming dark and visible but did increase in thickness. In 1930 infection was obtained throughout the summer and as late as October 18. Apples inoculated in early October developed a lighter type of infection than that produced on apples inoculated during June, July, and August.

The fungus was reisolated from the infected apples in many of the tests and proved to be identical with the original culture from which the inoculum was taken.

INOCULATIONS IN THE ORCHARD

Sooty blotch was produced on inoculated apples in the orchard during the season of 1930, when no natural infection was observed either on the 1,375 uninoculated apples used as controls or on apples in neighboring orchards and the commercial crop. The apples were inoculated during a rain or the period of high humidity immediately following a rain by atomizing with a spore suspension taken from the cultures. In all of the inoculations except those on 1 tree, the inoculated apples were not bagged or protected in any way. No spray was applied to the apples after the calyx application, except that certain trees, as indicated in table 2, were sprayed with lime sulphur 1 to 50 on July 10.

In 1930 sooty blotch was produced on 6 varieties of apples in the orchard (Table 2) by inoculating with spores from cultures from sooty blotch on apple, papaw, and white ash on June 6, 7, 11, 16, 17, and 30. No infection was obtained by inoculations with spores from a culture from hawthorn, although this was pathogenic in the moist chamber. The severity of the infection varied from a single small thallus to numerous colonies coalescing to form large, black, dense, irregular blotches partially covering the apple, and no differences in the pathogenicity of the cultures from apple, white ash, and papaw were noted. Reisolations of the pathogenic cultures were made. No infection was secured on the early varieties Yellow Transparent, King David, Maiden Blush, and Wealthy, all of which were harvested on

or before August 26. Inoculations made on Grimes, August 5, had not developed infection on September 19, the date of harvesting. It may be that infection took place on these early varieties but did not have sufficient time to become visible under the existing conditions.

Only about 5 per cent of infection was obtained from inoculations made on June 11 on apples later sprayed with lime sulphur 1-50 on July 10,

TABLE 2.—Results of inoculation of apples in the orchard, with spores from cultures of sooty blotch, 1930 and 1931

Date in- oculated	Source of culture	Tree No.	Variety in- oculated	Number fruits in- oculated	Date in- fection noted	Percentag infected
6–6	Apple	1	Rome	140	9-1	26a
6-6	7.7	2	Jonathan	128	8-26	64a
6-6	White ash	2	"	121	8-20	35
6-6	Papaw	2	"	290	8-26	60
6-6	Hawthorn	2	1 CC 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	120		0
6-7	Apple	3	Seedling	150	8-26	50a, b
6-7	77	4	King Davide	193		0
6-11	"	5	Jonathan	48	9-1	38
6-11	"	6	Grimes	76	9-1	60a
6-11	"	7	Deliciousd	269	9-11	5b
6-11	"	8	Bismarckd	190	9-11	5b
6-11		9	Bertha	89	8-21	34.8
6-11	Bladder nut	9		100	8-21	2
6-12	Apple	10	Delicious	10	8-21	100a
6-12	Willow	10		55	8-21	78a
6-12	Sycamore	11	Bismarck	135	8-21	24a
6-12	Apple	12	Senator	15	8-21	40
6-12	Leatherwood	12	""	95	8-21	44a
6-12	Grape	12	"	60		0
6-12	Apple	13	Satsuma	30	8-21	53
6-12	Red elm	13	"	125	8-21	1
6-12	Hawthorn	14	Greenville	60		0
6-12	Blackberry	14	66	75		0
6-12	Apple	15	Salome	98	8-21	55
6-12	Smilax	15		185	8-25	18a
6-12	Maple	15	"	105	8-21	19
6-12	Apple	16	Rome	103	8-21	58
6-12	White ash	16		98	8-21	70
6-16		17	Satsumad	80	9-19	5b
6-17	Apple	17	a c c d	243	9-11	10b
6-17	Papaw	17	cc d	60	9-11	5b
6-30	Apple	18	Romed	300	9-11	5b
7-13	7.7	19	"	133	8-21	52
7-13	White ash	19		75	8-21	53
7-19		20	"	257	8-21	80
7-19	Apple	20	"	125	8-21	90
8-3	7.7	21	Black Twig	240	9–2	85
8–3	White ash	21	" "	264	9-2	92
8-5	Apple	22	Grimes	250	· -	0

a Sooty-blotch fungus reisolated.

b Estimate. Fruit picked before counts were made.

c Fruit picked August 26, 1930.

d Fruit sprayed July 10 with lime sulphur 1-50.

while on apples not receiving the spray 38 to 60 per cent of infection was obtained. This decrease in the percentage of infection on the sprayed fruit was probably the result of the fungicidal action of the spray applied on July 10.

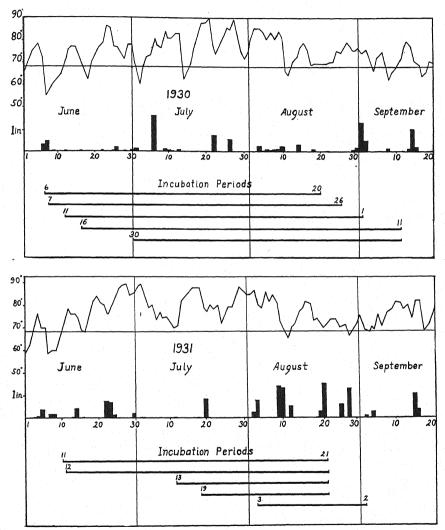


Fig. 1. Incubation periods of sooty blotch in orchard inoculation tests and the weather conditions in 1930 and 1931. The horizontal lines represent the incubation period between date of inoculation and date when sooty blotch became visible. The curve represents the average daily temperature and the vertical columns the rainfall in inches (from Climatological Data). The optimum temperature for the fungus, 68° F., is indicated.

The infection on apples inoculated on June 6, 7, 11, 16, and 30, 1930, did not become visible until after August 20 and the incubation periods were 75, 80, 82, 87, and 73 days, respectively (Fig. 1). The long duration of the incubation period may have been the result of the very hot and dry summer.

During the period of inoculation in June, 1930, conditions were favorable for infection, but the climatic conditions during July, except for a cool period of 3 days, were not favorable for the development of the fungus, which grows very slowly at a temperature above 80° F. and a humidity less than 90 per cent, as will be shown later. In August, 1930, rainfall occurred during a period from the 4th, to the 10th, again on the 14th and on the 18th, and the high temperature (90–100° F.) of the 1st 9 days was followed by lower temperatures (80–90° F.) during the remainder of the month and throughout September. The lower temperature and high humidity of mid-August favored the development of the fungus, which first became evident on the apples as small dark sooty blotches on August 20. As in the moist-chamber inoculations, it was noticed that there was little or no increase in the size of the colonies after they turned dark and became visible; consequently, their early stages of development were not observed.

Inoculations were made in the orchard again in 1931 with a number of cultures not previously used. In all but 2 of the tests parallel inoculations were made with a culture of proved pathogenicity from apple. In 1931 sooty-blotch infection was observed on apples in neighboring orchards and also on the commercial crop. In the inoculation tests, however, the 600 noninoculated apples used as controls remained free from infection. In 1931 sooty blotch was produced on 8 varieties of apples in the orchard (Table 2) by inoculations on June 11, 12, July 13, 19, and August 3 with spores from cultures of sooty blotch from apple, bladder nut, papaw, willow, sycamore, leatherwood, red elm, smilax, hard maple, and white ash. Reisolations were made from the apples inoculated with the cultures from apple, willow, sycamore, leatherwood, and smilax.

As compared with the culture from apple, some of the cultures showed much lower percentages of infection. For example, no infection was obtained with the cultures from grape and hawthorn, and the cultures from red elm, smilax, hard maple, and sycamore produced low percentages of infection, although all of these cultures had produced abundant infection in the moist chamber. The culture from blackberry was nonpathogenic in the moist chamber as well as in the orchard.

The first evidence of infection in 1931 on apples inoculated on June 11, 12, and July 13 and 19 was noted on August 21 (Fig. 1), while apples inoculated on August 3 developed the first evidence of infection on Septem-

ber 2, 30 days after inoculation. Thus, the period between the date of inoculation and of evident infection decreased from 71 days early in the season to 30 days late in the season.

THE RELATION OF CLIMATIC CONDITIONS TO INCUBATION PERIOD

The relation of climatic conditions to the incubation period for the orchard inoculations made at different dates is shown in figure 1. In 1930 the incubation periods were all long, owing probably to the high temperatures and light rainfall of June and August, and the inoculations made on June 16 showed up no sooner than those made on June 30. In 1931 there was more rain in June and August, though less in July, and infection from inoculations made on June 11 and 12 showed up no sooner than from those made a month later. Apparently, the high rainfall and lower temperatures of August favored the development of the sooty-bloth colonies. The progressive shortening of the incubation period with each later inoculation in 1931 suggests that cool moist conditions late in the summer favor the development of the colonies. Because the fungus grows best at a fairly low temperature (68° F.) and almost none at all at 86° F., (midsummer is usually too hot in this region for its best growth) it is suggested that most of the growth is made in June and August. Since high humidity also is necessary for the growth of the fungus, as will be shown later, it is probable that rainfall is more important during these months than in July. This might account for the much heavier natural infection in 1931 than in 1930. The heavy dews that occur in August no doubt also favor the development of the fungus.

Since the spores are mature in late May and early June it seems likely that in nature the fungus becomes established on the fruit early in the season. Apparently, however, its development may be very slow or completely suspended during hot, dry weather, although the small colonies may be present in a colorless invisible condition. With the advent of lower temperature and higher humidity in August, the colonies probably enlarge and become pigmented and visible.

THE SOOTY-BLOTCH FUNGUS IN CULTURE

On potato-dextrose agar the colonies of the fungus isolated from apple are slow growing and form a dark, dense, compact, leathery thallus, which piles up and extrudes masses of spores promptly and in abundance (Fig. 2, E). The older mycelium bears numerous short lateral hyphae branching mainly at right angles to the main hyphae. The spores are produced in cavities at various levels in the thallus, break through the dense layer of mycelium, and ooze out on the upper surface of the thallus and around its

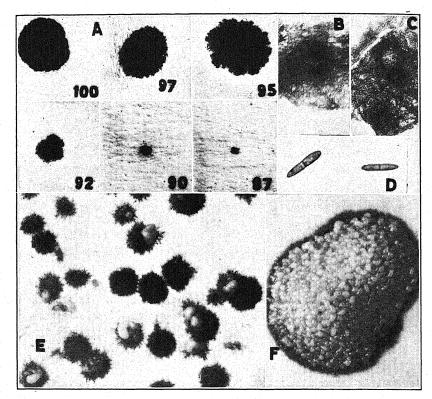


Fig. 2. A. Growth of sooty-blotch colonies on filter-paper pads with potato-dextrose nutrient solution after 2 months under humidities of 87 to 100 per cent. The fungus grew well at humidities above 92 per cent. B and C. Sooty-blotch pycnidia on blackberry: B shows radial cracks in the ruptured top; C shows the sides forced wide apart and the spore mass within. X 170. D. Spores from a culture of sooty blotch from apple. X 375. E and F. Sooty-blotch fungus on potato-dextrose agar. E. 14-day-old sporulating colonies on a poured plate inoculated with spores from a culture of sooty blotch from apple. The fungus produces a dense black thallus on the surface of which the spores ooze out in gelatinous masses. X 3. F. 18-day-old colony of sooty blotch from willow with the surface nearly covered with the spore masses. X 3.

margin in gelatinous masses that may entirely cover it (Fig. 3, B). When first extruded the spore masses are whitish or pinkish, but later turn dark. When the thallus has dried somewhat and drawn up to form a hollow mound above the agar, the spores may be extruded onto the agar underneath it.

The spores germinate readily by elongating at both ends, and it is rather difficult to find very many ungerminated spores in the spore masses produced in culture. In spore smears on moist fresh agar, the spores bud and proliferate directly, producing a pinkish yeast-like type of growth. In the

later stages of development or when the substratum dries, the characteristic dark colony may be produced. Isolated spores on poured plates of agar, however, characteristically produce the black mycelial colonies.

Cultures from the other hosts on potato-dextrose agar are, in general, similar in type of growth and sporulation to those from apple, but some differences have been observed. Groves (4) has reported differences in cultural characters among the various sooty-blotch isolations. In order to determine whether there were differences between the cultures from different hosts, the cultures from 17 hosts were compared on 13 different agar media in Petri dishes at 24° C. With all of these cultures, except those from blackberry and dogwood, infection had been obtained on apples.

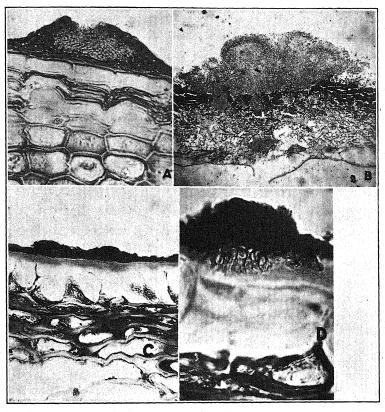


Fig. 3. Cross-sections of sooty blotch. A. Pycnidium on leatherwood. X 530. B. Young sporulating colony on potato-dextrose agar. Spores are produced in cavities at various levels in the thallus and break through to the surface in gelatinous masses. X 14. C and D. On Grimes apple. C. Dense layer of mycelium on surface of cuticle. X 345. D. Short hyphae, usually in clusters, penetrating only a fraction of depth of cuticle. X 960.

On all of the media the cultures from apple, papaw, hawthorn, leatherwood, bladder nut, hard maple, white ash, smilax, sassafras, sycamore, spice bush, prickly ash, and dogwood were more or less alike and produced dark, heaped-up colonies on potato-dextrose agar with little or no aerial mycelium and spore masses varying from sparse to abundant. On oatmeal agar the colonies were prostrate and did not sporulate. The cultures from willow (Fig. 2, F), red elm, and blackberry differed in that the colonies on potato-dextrose agar were prostrate and sporulated more abundantly. The colonies from the blackberry culture had a whitish margin. The culture from grape was of lighter color, produced more aerial mycelium, grew more rapidly than the others, and sporulated on oatmeal agar.

In order to determine the suitability of various sources of carbon for the fungus, a culture of proved pathogenicity from apple was grown on agar media containing as a carbon source, dextrose, levulose, sucrose, maltose, lactose, malt extract, dextrin, or potato starch in concentrations of 2, 1, 0.5, 0.1, and 0.05 per cent. Potato extract in concentrations of 20, 10, 5, 1, and 0.5 per cent also was used as a carbon source. At the higher concentrations of all the carbon sources the sooty-blotch fungus made a good growth, somewhat better on levulose, sucrose, dextrin, potato starch, and potato extract than on the other carbon sources. Spores were produced on the malt-extract agar in all concentrations and in the 3 higher concentrations of potato extract, but not on the other media.

The rate of growth of the fungus on 5 kinds of nutrient agar was determined by measuring the diameter of the colonies after 7 and 15 days at 24° C. The growth was most rapid on prune agar, the colonies measuring 7.1 mm. in diameter at 7 days and 11.6 mm. at 15 days. On potato agar with 2 per cent dextrose, the diameter was 5.1 mm. at 7 days and 8.6 mm. at 15 days, and on Leonian's synthetic agar (5, p. 762) the measurements were 4.6 mm. and 7.0 mm., respectively. On these 3 media the colonies were heaped-up and produced spores. The poorest growth occurred on corn meal and oatmeal agar, the colonies were prostrate, and no spores were produced. On corn-meal agar the diameter at 7 days was 4.2 mm. and at 15 days, 8.0 mm. On oatmeal the corresponding dimensions were 4.3 mm. and 7.7 mm.

EFFECT OF TEMPERATURE, HUMIDITY, AND H-ION CONCENTRATION ON GROWTH OF THE FUNGUS

The temperature range for the growth of a culture from apple of proved pathogenicity was determined by measuring the increase in diameter of the colonies in Petri dishes on potato-dextrose agar at temperatures between 0 and 30° C., maintained within a degree. The average increase in diameter of the colonies in Petri dishes on potato-dextrose agar at temperatures between 0 and 30° C., maintained within a degree.

eter of the colonies after 7 days was 0.4 mm. at 0°, 1 mm. at 6°, 2.8 mm. at 10°, 2.6 mm. at 13°, 3 mm. at 15°, 3.4 mm. at 18°, 6.9 mm. at 20°, 5.3 mm. at 27°, 0.4 mm. at 30°, and after 14 days was 1.1 mm. at 0°, 1.9 mm. at 6°, 4 mm. at 13°, 7 mm. at 18°, 10.6 mm. at 20°, 9.4 mm. at 24°, 8.6 mm. at 27°, and 1 mm. at 30°. Therefore, growth was most rapid at 20° and was good between 18 and 27° C. Almost no growth occurred at 0 or 30° C. The virtual failure of the fungus to grow at 30° C. (86° F.) may explain its slow development in hot weather, and the low optimum of 20° C. (68° F.) explains why it is favored by cool weather.

For use in testing the effect of humidity on the growth of the fungus, filter-paper pads were saturated with a 2 per cent potato-dextrose nutrient solution, placed in small preparation dishes, and sterilized. These preparation dishes were then placed inside of larger ones, containing different concentrations of salt solution to control the humidity. The filter-paper pads were allowed to remain over the different salt solutions for 4 days, so that equilibrium in vapor pressure was presumably established between the paper and the salt solution. According to Lesage (6), the humidity over a salt solution varies inversely with the concentration of the salt solution. According to his formula, the following humidities were provided: 97 per cent (over 5 per cent NaCl), 95 per cent (8.3 per cent NaCl), 90 per cent (16.6 per cent NaCl), 87 per cent (21.6 per cent NaCl), and 85 per cent (25 per cent NaCl).

The fungus was planted in the center of each filter-paper pad by touching with a platinum needle covered with spores from the gelatinous spore mass of a culture. Five colonies were grown in each humidity. The average increase in diameter of the colonies after 2 months was 15.5 mm. at a calculated humidity of 100 per cent, 11.1 mm. at 97 per cent, 12 mm. at 95 per cent, 5.7 mm. at 92 per cent, and 1.6 mm. at 90 per cent, and no growth occurred at humidities of 87 and 85 per cent. Therefore, the growth was best in the higher humidities (95 per cent and above) and rapidly became less with decrease in humidity (Fig. 2, A).

This test was repeated in a manner similar to that described above, except that the filter-paper pads were not placed over the salt solutions until 4 days after inoculation. At this time small black colonies were visible. The relation of humidity to growth was found to be about the same as in the previous test.

Since this fungus is superficial on its host, it seems safe to assume that atmospheric humidity will directly affect its growth and, consequently, that growth will occur only during periods of very high humidity.

On potato-dextrose agar adjusted to different hydrogen-ion concentrations, the average diameter of the colonies after 10 days at 24° C. was 4.1

mm. at pH 3.3, 4.5 mm. at pH 3.6, 7.3 mm. at pH 4.15, 6.5 mm. at pH 4.66, 7.4 mm. at pH 5.6, 7.5 mm. at pH 6.26, 8.4 mm. at pH 6.75, 8.6 mm. at pH 7.37, 6 mm. at pH 7.8, and 4.7 mm. at pH 8.3. Between pH 4.15 and 7.8 the fungus grew well, and its limits of tolerance were not reached in these tests. Apparently, it tolerates a very wide range of H-ion concentration.

MORPHOLOGY OF THE FUNGUS

In sections of apple-fruit tissue of the Grimes variety embedded in nitrocellulose and staimed with safranin, the fungus was observed to be superficial (Fig. 3, C), as Colby (1) reported, but with occasional clusters of short hyaline hyp has extending slightly into the cuticle (Fig. 3, D). These clusters of short hyphae were usually under the immature pycnidia and penetrated only a fraction of the depth of the waxy cuticular layer. Groves (4), however, has found types of sooty blotch that penetrate beneath the cuticle.

The morphology of the fungus has been described by Colby (1). Mature pycnidia, as found on twigs in May and June, 1931, were dark brown to black, dimidiate, scattered and superficial. The pycnidia varied in size, those on leatherwood (Fig. 3, A) were 60 to 130 μ in diameter, 20 to 40 μ in height, with a cavity 30 to 87 μ in diameter. On apple fruit, where only immature pycnidia have been found, they were 85 to 140 μ in diameter. On blackberry the pycnidia were 60 to 130 μ in diameter, on bladder nut, 61 to 105 μ , and on hard maple 85 to 130 μ . The top of the pycnidium is ruptured irregularly near the center by the formation of radial cracks (Fig. 2, B and C) and is forced wide open, apparently, by the pressure of the mass of spores within. The flaps formed by the radial cracks fall back over the empty cavity after the spores are discharged, leaving a stellate opening.

The immature spores are unicellular, but the mature ones, found in abundance in late May and early June, 1931, were bicellular, sometimes tricellular, hyaline, slightly pointed at the ends and measured $2\,\mu$ x 10–12 μ . These spore measurements agree well with Floyd's (2), but Colby (1) found the spores to be larger, and both found them to be unicellular.

In culture the spores are produced in numerous cavities apparently without distinct walls, varying in size, and located at different depths in the thallus (Fig. 3, B). The spore masses ooze out in abundance on top of the thallus and around its margin (Fig. 2, E). The spores produced in culture (Fig. 2, D) are slightly larger than those produced on the host, measuring 2–3 μ x12–14 μ ; but, as previously mentioned, they start to grow so promptly in their gelatinous matrix by elongating at the ends and producing additional septa that ungerminated spores are not easily found in the cultures, and measurements of such spores are not reliable.

While we have not observed sporulating pycnidia on apple the fact that the spores from the pycnidia of sooty bloth on 10 other hosts were bicellular and that the spores produced in cultures from apple as well as the other hosts were, likewise, bicellular would indicate that the sooty-blotch fungus, *Gloeodes pomigena*, is characterized by bicellular rather than unicellular spores.

SUMMARY

The sooty-blotch fungus has been isolated in pure culture from apple and crab-apple fruit and from the young twigs of 23 other host species by means of tissue plantings or poured plates inoculated with spores from pyenidia on the hosts.

Pycnidia with mature spores were found in late May and early June on the twigs of 12 host species. The spores were liberated early in June.

The mature spores are bicellular.

In a cool moist chamber, in the greenhouse, green apples were inoculated with spore suspensions and infection was obtained with the cultures from apple, papaw, white ash, sycamore, smilax, hard maple, willow, bladder nut, leatherwood, red elm, prickly ash, hawthorn, spice bush, grape, and sassafras. No infection was obtained with cultures from blackberry. The incubation period was 2 to 3 weeks.

In the orchard, sooty blotch has been produced on apples by inoculation with spores from cultures from the first 10 host species mentioned above. The incubation period varied from 1 to 2 months. The dry, hot weather of midsummer is unfavorable to the fungus.

Under the superficial thallus on the apple cuticle, clusters of short hyphae are found, penetrating a short distance into the cuticle.

The fungus grew well on a wide variety of agar media. Spore production in culture was favored by malt and potato extracts. Minor differences in cultural characters were noted. The fungus grows slowly and, on potato-dextrose agar, produces a more or less heaped-up, black, leathery colony and an abundance of spores that coze out in gelatinous masses.

The optimum temperature for growth was about 20° C. Good growth occurred between 18 and 27° C. Practically no growth occurred at a humidity of 90 per cent or less. A wide range of H-ion concentration was tolerated.

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THE MECHANISM OF VARIATION IN IMPERFECT FUNGI: BOTRYTIS CINEREA

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INTRODUCTION

The subject of variation or subspecific grouping in fungi has been reviewed by Brierley in 2 recent papers (3, 4). Reference is made to these publications for a broad discussion of matters relating to the present topic.

The cosmopolitan gray-mold organism, Botrytis cinerea Pers. (B. vulgaris Fr.), furnishes an excellent example of a fungus species of a type that is of particular interest in the present connection. It is a common observation that much variation exists between different collections or isolations of fungi that pass for this species and that, in a general way, show a fair degree of uniformity. The uncertainty of specific limitations in such material seems to increase almost indefinitely with the multiplication of cultures, either from new material or from previous isolations.

Brierley's conception of the situation with this particular fungus is expressed as follows: "The species Botrytis cinerea may be visualized as, at any one moment, a cluster of numerous races or strains morphologically congruent on the host plant but in vitro showing marked and constant cultural differences." The same author points out the fact that since variants with morphologically or culturally distinct characters are so numerous there probably also exist physiologic strains that may or may not be morphologically alike. The manner of origin of the apparently numberless strains of this and other supposedly asexually reproducing fungi is a subject of much interest. The principal explanations that have previously been advanced for this condition are as follows:

Impure Material. This theory assumes that such fungi as Botrytis cinerea are composed of complex mixtures of established types and that repeated culturing from this complex by single-spore isolations or mass transfers results simply in separating from the mass either single strains or new combinations of strains ad infinitum. In case single-spore strains continue to vary, suspicion is directed at the technique of single-spore isolation. This explanation does not, of course, account for the original formation of these diverse strains.

Mutation. That variation goes on in many vegetatively propagating (asexual) fungi, even in strains originally established from unquestionable single-spore isolations, has been shown by many workers. The term "mutation" (saltation, dissociation, discontinuous variation) has been

rather loosely applied to characterize this phenomenon. (See discussion by Stakman et al., 12, p. 53.) The extraordinary degree to which new variants appear in certain fungi has led others to seek some explanation of the remarkable behavior of these organisms more in keeping with the conventional principles of genetics (Brierley, 4). Meantime the need of far more cytological evidence and genetic analysis is evident and forms the motive of the work presented in this paper.

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Hybridization. The existence of a more or less frequently occurring, though undiscovered, perfect or sexual stage of the fungus, making possible hybridization between species or strains, with heterozygosis and ordinary segregation in the offspring, has been suggested as an explanation of variation in fungi. Although no form of sexual fusion that could bring about hybridization, character combination, and segregation is ordinarily recognized in the so-called imperfect fungi per se, the fact should not be overlooked that Botrytis cinerea has, theoretically at least, a perfect, ascomycetous stage, represented by the species Sclerotinia fuckeliana de Bary. Although the apothecial form of the latter fungus is common in Europe on dead vine leaves, it has never, so far as we are aware, been reported in America, and considerable doubt prevails as to the relation between these 2 fungi. The original announcement in the matter by de Bary (1) certainly indicates uncertainty. Its confirmation by Pirotta (8) and Istvanffi (6), and the observations of Viala (14), Prillieux (9), Ferraris (5), and other European writers on vine diseases are hardly more convincing. Tubeuf (13) indicates doubt whether "S. fuckeliana and B. cinerea are stages in the life of the same fungus," although he has "observed the Peziza fruit, so easily cultivated from sclerotia gathered in the open air (e.g., from vine leaves)." One of us (Smith, 10) concluded, on the basis of rather extensive work in America and Europe, that "so far as known no Peziza has ever been obtained from sclerotia developed from Botrytis." Brierley (3) states, "Many years' study of B. cinerea has given me no evidence of a connection with Sclerotinia."

H. H. Whetzel, in a personal letter, expresses the opinion from his own observations and studies that all Botrytis forms of the *cinerea* type are conidial stages of apothecial species belonging to the genus Sclerotinia, as that genus is now generally understood. He has established the connection between the apothecial form and the Botrytis stage of several species of Sclerotinia and has been able to confirm the connection between S. fuckeliana and the common Botrytis on grapes referred to by de Bary as B. cinerea. This statement is based upon his as yet unpublished researches. From all the evidence it appears to be true that there occurs commonly on vines, in southern Europe at least, an apothecial, Botrytis-associated Sclerotinia fungus that has not been found in America and apparently not in England.

Kharbush (7) reports the usual phenomena in the development of the ascogenous hyphae and asci in *Sclerotinia fuckeliana*.

Whether or not *Botrytis cinerea* develops an apothecial stage in California is, in fact, immaterial in the present connection if conidial reproduction be considered as representing purely asexual or vegetative multiplication of clones. Should crossing of strains and the development of a hybrid apothecial stage actually occur, resulting in heterozygosity, there would still be no provision for segregation or dissociation and for the origin of new strains in these conidial lines after their original development from ascospores. Whether, in fact, the formation of conidia from conidiophores or pycnidia in *fungi imperfecti* is as purely an asexual, vegetative reproductive process as the multiplication of higher plants by cuttings or stolons is a question that cannot be too positively answered in the affirmative until more cytological evidence is available.

Microconidia. The occurrence of specifically distinct bodies of this sort in *Botrytis cinerea* may have some possible significance, although there is little evidence that they have even enough power of germination to play any important rôle.

Anastomosis. Hyphal Fusions. Heterocaryosis. Mixochimaera. That anastomoses or fusions often take place between hyphae and germ tubes of many fungi,¹ affording a possibility for the interchange of cell contents, was figured and described by de Bary (1) and by many others since that time. The frequently occurring multinuclear condition in hyphal cells and spores of such fungi has suggested the possibility of an irregular distribution of genetically dissimilar nuclei in cell division or conidial formation. This phenomenon has been the basis of considerable speculation in relation to the origin of variation in fungi, although no actual, cytological evidence in the matter has been produced (see Brierley, 2, 3, 4).

CYTOLOGICAL STUDIES

Cytological studies carried on in connection with this work showed that the mycelial cells and conidia of Botrytis are multinucleate, containing comparatively large numbers of nuclei. Spores of the a homotype hereafter described contain from 6 to 18 nuclei and those of the b homotype from 3 to 9 nuclei, with much greater variation in the mycelial cells. Figure 1 shows the nuclei in hyphae and germinating conidia and also anastomosis between neighboring filaments. In D and E nuclei can be seen in the connecting strands and, in E, a hypha has arisen from this point. Anastomosis takes place by lateral fusions of hyphae near the growing tips or by the

¹ The term "heterocaryosis" precisely describes the condition of a cell containing 2 or more genetically different nuclei and, for this reason, its use seems preferable to "mixochimaera."

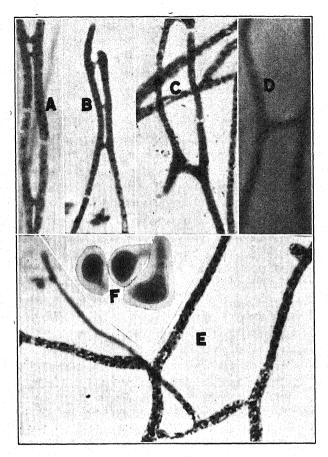


Fig. 1. A-E. Anastomosis in *Botrytis cinerea*. Note multinuclear condition of cells. F. Multinuclear germinating conidia.

pushing out from the sides of older filaments of new lateral hyphae, which fuse terminally with the tips of other similar hyphae or directly with the cells of neighboring hyphae. Fusion also may occur between the germ tubes of 2 conidia. Nuclear fusions were not observed.

ISOLATION OF TYPES OF BOTRYTIS

In connection with work on *Sclerotinia sclerotiorum* recently published by one of us (Smith, 11) 47 mass-transfer cultures of Botrytis were made from various samples of vegetation that had been obtained in different parts of California and incubated in moist chambers. The original cultures were mostly of the general type of *Botrytis cinerea*, although varying somewhat from each other, and, in some cases, mixed with other fungi. Reculturing

several times by mass transfer of small quantities of conidia to eliminate foreign fungi resulted finally in a series of apparently pure cultures of Botrytis showing considerable variation in type of mycelium, conidial production, presence, size and character of sclerotia, and other gross characters. Four monospore cultures were then made from each of the 47 strains. In some the 4 cultures looked identical, others showed decided variation. The series as a whole showed a wide range of variation. Many of the strains, in fact, might easily have been regarded as distinct species if the intermediate types had not been seen. This work clearly showed that (1) B. cinerea collected from different places in California has much variation and (2) single-spore isolations from apparently homogeneous mass-transfer colonies reveal further variation, so that the definition of specific limits is extremely difficult.

Further Analysis of Types of Botrytis cinerea by Single-spore Cultures. In order to study further the matter of variation in this fungus it was decided to make repeated monoconidial cultures of various strains for a number of generations, rather than follow the usual procedure of successive mass transfers of strains that had been subjected to single-spore culturing only in the original isolation.

METHODS

When single-spore cultures are to be made in large numbers (Fig. 2) represents 1,830 cultures) it becomes necessary to use an isolation method that is both precise and expeditious. Our technique was as follows: the approximate concentration of a spore suspension was determined by direct microscopic counts of spores carried in a 2-mm. loop. From this suspension 75 to 100 spores were transferred to a test tube containing 10 cc. of melted Czapek's medium made with 0.5 per cent agar. The low percentage of agar makes it possible to cover the bottom of 3 90-mm. plates with 10 cc. of medium, giving from 20 to 40 spores to each plate. The thinness of the film of agar greatly minimizes the possibility of spores being situated directly above one another. The spores were now allowed to germinate for from 12 to 16 hours, during which time they developed germ tubes from 5 to 10 times their own diameter. The Petri dish was then placed on the stage of a dissecting microscope with a magnification of about 35, the cover removed, and the spores picked up singly on the tip of steel needles and transferred to agar slants. The needles used were made from steel knitting needles ground down to a very fine point, the terminal millimeter tapering from 250 u to 10 u in diameter at the tip. Such a tip can readily be inserted under a germ tube and the entire spore lifted out. A needle of such fineness must be sterilized by chemical means, as it is immediately destroyed in a flame. All cultures were grown on potato-dextrose-agar

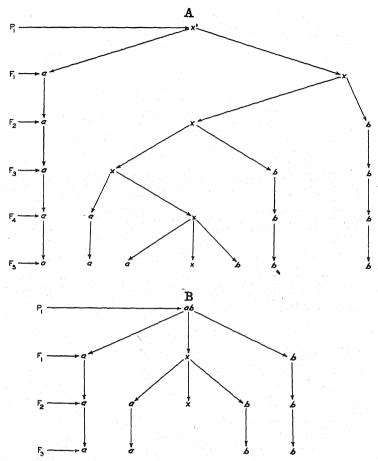


Fig. 2. A. The behavior of the progeny of a single conidium of $Botrytis\ cinerea$ (strain x^i) through 5 generations, showing the separation of the homotypes a and b and the appearance of heterotypes x. The chart represents a total of 650 single-spore cultures. B. Behavior of the combination of strains a and b of $Botrytis\ cinerea$ through 3 generations. The chart represents a total of 1,180 single-spore cultures.

slants in large test tubes 8×1 in., at room temperature for 10 days, at the end of which time records were taken and the next generation of single-spore cultures started.

From the 188 single-spore Botrytis cultures available, there were selected 8 of the *cinerea* type that represented the greatest morphological variation. From each of these, 25 single-spore cultures were made. Many of these showed almost absolute uniformity throughout the 25 tubes. Some showed quite striking variation. Figure 3, A and B, shows 2 tubes, each, of these 8 strains. From 1 tube of type 8 (Fig. 3, B, the 2 tubes at right

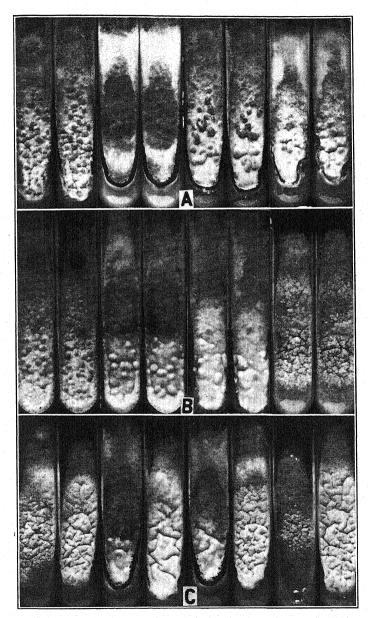


Fig. 3. A and B. Eight strains of Botrytis isolated by single-spore cultures from various collections; 2 cultures of each strain (1-8). C. Eight single-spore cultures from strain 8 in B, showing the variation that occurred after several generations of single-spore culturing.

end) (a strain in which variation occurred) 25 monospore cultures were made. Much variation appeared in these cultures, 8 of which are shown in figure 3, C.

From another of the original single-spore cultures from 1 of the 47 strains (marked x^1) 150 monospore cultures were grown. Of these, 143 looked alike and were labelled a, while the remaining 7, labelled x, differed materially from the a type in amount and color of mycelium, abundance of spore formation, and in other gross features (Figs. 2, A and 4). From

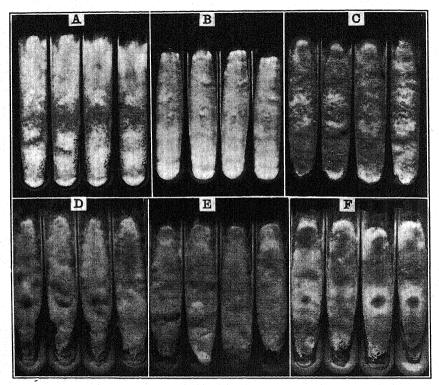


Fig. 4. A. Homotype a of Botrytis cinerea. B. Homotype b. C-F. Heterotypes x.

one of the a type tubes 25 single-spore cultures were grown, all of which were of the a type. Succeeding generations of a remained true to type (Fig. 2, A). From one of the x-type tubes in the F_1 25 monoconidial cultures separated into 7 of x and 18 of an entirely new type, b (Figs. 2, A and 4), with short, dense felt-like, yellow mycelium, quite unlike any other strain. The latter remained true to type through succeeding monospore generations (Fig. 2, A). In F_2 , F_3 , F_4 , and F_5 the x types separated into x and x types, x and x types, or into x, x and x types (Fig. 2, A). The

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net results so far, summarized in table 1 and figure 2, A, are the appearance of 2 homotypes, a and b, and several heterotypes x, the heterotypes separating into heterotypes and homotypes and the homotypes remaining constant. This behavior strongly suggested that the x heterotypes were composed of various proportions of the 2 homotypes. According to this

					Pro	geny		
Genera- tions	Parents	No. of cultures	x	1	ъ			
			No.	%	No.	%	No.	%
P ₁	$x^{_1}$	150	7	4.7	143	95.3		
F ₁	\boldsymbol{x}	25	7	28.0			18	72.0
	а	25			25	100.0		
$\mathbf{F_2}$	x	25	18	72.0			7	28.0
* 1	a	25			25	100.0	05	1000
	Ъ	25					25	100.0
F ₂	x	50	47	94.0	3	6.0		
	a	25			25	100.0		
	ь	50					50	100.0
F ₄	x	125	92	73.6	23	18.4	10	8.0
	a	75			75	100.0		
	ъ	50					50	100.0

TABLE 1.—Synopsis of single-spore cultures from strain x^1

suggestion, if the homotypes a and b could be combined into a heterocaryotic condition and their progeny analyzed by single-spore cultures, it should be found that their behavior would be similar to that of the progeny of x^1 with which we started. If such combination took place we should predict that 3 types would appear in the F_1 , namely, the 2 parental types a and b and a number of intermediate or heterotypes. If a and b did not combine but merely grew together as a mixture we would expect the F_1 progeny to consist of the 2 parental types only. An attempt was made to combine a and b of the F_1 by taking a small amount of mycelium and spores from each and mixing them thoroughly together in warm agar; when the agar hardened, small amounts were transferred to 4 agar slants. When these cultures were 10 days old 25 single-spore cultures were made from each, giving 100 cultures from the cross. Of the 100 cultures, 50 were of the a type, 8 of the b type, and 42 of the x heterotype. Half of the cultures from each type were carried into the F_2 . From each of these 50

 $^{^2}x$ designates a number of morphologically or culturally different types with only one thing in common, namely, their inconstancy.

tubes, 20 single-spore cultures were made with the following results (see Table 2 and Fig. 2, B). The 500 cultures from a all came true to type, as

TABLE 2.—Synopsis of	f sinale-snore	cultures from	combination of	strains a and b

			Progeny								
Genera- tions	Parents	No. of cultures		x a b							
			No.	%	No.	%	No.	%			
P ₁	ab	100	42	42.0	50	50.0	8	8.0			
F ₁	$oldsymbol{x}$	420	153	36.5	206	49.0	61	14.5			
	a	500			500	100.0					
	ь	80					80	100.0			
F ₂	а	20			20	100.0					
	ъ	60					60	100.0			

did also the 80 cultures from b, but the 420 cultures from the x heterotypes separated into a and b homotypes and several x heterotypes. Homotype b was also successfully combined with 2 (see Fig. 3, A, second pair from left), a strain from a different geographic locality, with essentially the same behavior of the progeny as in the ab combination.

DISCUSSION

The results obtained in the F_1 and F_2 of the cross ab indicate that fusion of some sort has taken place and that the intermediates or heterotypes are composed of various proportions of a and b. The regularity and completeness with which the homotypes separated from the heterotypes indicate that the character-determining elements are discrete units of limited number. This leads to the suggestion that the basic unit of the individual is the nucleus and not the cell. In other words, a multinucleate spore is not an individual but a group of individuals, a colony, and it will not give rise to a genetically pure culture unless all its nuclei are genetically identical. The number of variants that may arise from a spore having 2 kinds of nuclei will depend on the number of nuclei it contains. For example, if the sum of the 2 kinds of nuclei in a given spore were 8, such a spore with independent nuclear assortment could give rise to 9 variants, i.e., 2 homotypes and 7 heterotypes. Showing that genetically different nuclei can exist in the same cell would offer an explanation why monospore cultures so often give rise to variants, and we would then need only a mechanism for bringing together such diverse nuclei in the first place to account for variability in fungi imperfecti without attributing it in a vague sense to mutation. Such a mechanism has been shown to exist in the phenomenon of anastomosis. We have shown that in Botrytis cinerea the hyphal cells and conidia are multinucleate and that anastomoses between neighboring hyphae are very abundant, forming connecting strands whose cellular contents are derived from those of different filaments. In the mixed growth of strains that compose B. cinerea in nature anastomosis between different strains could hardly fail to occur, resulting in a mixture of nuclei from 2 or more strains in the same cell and eventually in the same conidium. If our observation is valid, that nuclei of 1 strain migrate into cells of another strain by means of anastomosis, it will mean that the genetic composition of a given cell may be quite different from that of contiguous ones, depending upon whether or not it had anastomosed and, if so, how many of the different nuclei had passed into it. It would seem that to demonstrate such a condition one would have to analyze the genetic composition, not of cultures, but of single cells, and this, in effect, is what we have attempted to do in the experiments reported in this paper. In addition, we have made single-spore analyses of Phoma terrestris, Verticillium alboatrum, Ramularia sp., and Fusarium sp. with results comparable to those above, indicating that heterocaryosis may be common in fungi imperfecti in general, and perhaps equally common in the conidial stages of fungi with perfect stages.

SUMMARY

- 1. Single-spore isolations from 47 cultures of *Botrytis cinerea* collected in various parts of California showed the existence of a large number of more or less dissimilar morphological strains.
- 2. Single-spore isolations were repeated through a number of successive generations of several of these strains. Under such procedure some of the strains remained uniform, while others continued to break up into further variations.
- 3. One of these strains (x^1) , which was subjected to single-spore culturing through 5 generations, gave rise to several types of cultures. Two homotypes (a and b) appeared that remained constant and several inconstant heterotypes (x) appeared that were intermediate. These latter, upon subsequent single-spore culturing, gave rise to other inconstant (x) forms and to the constant forms a and b.
- 4. Strains a and b were grown together in mixed cultures and their progeny analyzed by single-spore methods for 3 generations. In the F_1 and F_2 the parental forms a and b appeared and also a considerable number of inconstant types (x). The heterotypic strain x continued to produce heterotypes as well as the constant strains a and b.
- 5. The hyphal cells and conidia of *B. cinerea* are multinucleate, containing comparatively large numbers of nuclei.
 - 6. Anastomosis of hyphae is very common in this species.

- 7. It is suggested that by the mechanism of anastomosis nuclei of 1 strain may migrate into the cells of other strains and thus give rise to cells and spores containing 2 or more kinds of genetically different nuclei.
- 8. It is suggested that the basic unit of the individual is the *nucleus* and not the *cell* and that a multinuclear spore is, therefore, not an individual but, in reality, a colony and it can, therefore, not give rise to a genetically pure culture unless all its nuclei are genetically identical.
- 9. From the above it is concluded that variable forms of fungi imperfecti may owe their instability, not to mutation, but to nuclear heterogeneity (heterocaryosis), that this condition can be brought about both in vivo and in vitro by nuclei of one strain entering the cells of another strain through anastomoses, and that the reassortment of these diverse nuclei is accomplished by the mechanism of anastomosis and unequal cell divisions.

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MOSAIC DISEASES OF TOBACCO: V. DECOMPOSITION OF THE SAFRANIN-VIRUS PRECIPITATE

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INTRODUCTION

A number of procedures have been found applicable to the purification of the virus of mosaic disease of tobacco. For instance, Vinson¹ reported precipitation of the virus by means of acetone, alcohol, ammonium sulphate, and safranin. Moreover, Vinson and Petre² have reported that they obtained a high degree of purification by precipitating the virus from juice of diseased tobacco plants with a solution of lead acetate, then removing the virus from the lead precipitate by elution with a neutral phosphate solution. This latter method effects a remarkable purification of the virus, but the procedure is time-consuming and laborious. Brewer, Kraybill, Samson, and Gardner³ report the use of the ultracentrifuge and aluminum hydroxide in the purification of the virus.

Safranin in aqueous solution precipitates the virus from juice of diseased tobacco plants.⁴ This precipitate may be centrifuged off, as it packs fairly well, thus permitting decantation of the supernatant liquid. Vinson and Petre⁴ have described also a method of decomposing this precipitate and recovering the activity in the aqueous phase. The procedure is somewhat long and laborious, necessitating the subjection of the virus to a relatively high hydrogen-ion concentration.

Considerable care must be exercised in order not to inactivate the virus in the process. The original method of decomposing the safranin precipitate was not effective in eliminating the accompanying brown pigment, although there was a remarkable reduction in solids. The method of purification by precipitation with safranin was temporarily abandoned on account of the brown pigment that was not removed in the procedure.

Hope was never entirely abandoned, however, of eventually decomposing the safranin precipitate in such manner as to overcome the above-mentioned objectionable features. Such a method is here reported.

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- ³ Brewer, P. H., H. R. Kraybill, R. W. Samson, and M. W. Gardner. Purification and certain properties of the virus of typical tomato mosaic. Phytopath. 20: 943-950. 1930.
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EXPERIMENTAL PROCEDURE

Early in the work on the virus problem it was learned that juice from diseased plants could be treated with Lloyd's alkaloidal reagent without reducing the infective power of the treated juice. Safranin, being a base, it was recognized that Lloyd's alkaloidal reagent might combine with it. Consequently, it was found that this reagent would remove safranin from aqueous solution. It was then discovered that the same reagent would remove safranin from an aqueous suspension of a safranin-virus precipitate, leaving an aqueous phase with infectivity equal to, and often greater than, that of the starting material.

Tops of diseased Turkish tobacco plants are frozen, then thawed and the juice is expressed with a hydraulic press. The expressed juice is centrifuged at about 3,000 r.p.m. for 15 minutes to free it of the larger suspended particles. If the juice is obtained from plants grown in the greenhouse, 5 cc. of a 1 per cent aqueous safranin solution will precipitate the virus from 25 cc. of juice. If the juice is from field plants, 10 or even 20 cc. of the 1 per cent aqueous safranin may be necessary to complete the precipitation. The safranin is added to the juice in a centrifuge tube, and, after mixing, the tube is allowed to stand for a few minutes, then centrifuged at 3,000 r.p.m. for about 10 minutes. The red precipitate packs sufficiently to allow complete decantation of the supernatant liquid. The precipitate is then washed, using a volume of redistilled water equal to the volume of the original juice sample.

The precipitate from 25 cc. of juice, after washing, is suspended in 25 cc. of redistilled water, and 1 gm. of Lloyd's alkaloidal reagent is added to the suspension. The sample is thoroughly mixed, then allowed to stand for about 30 minutes, mixing at intervals. At the end of this time the sample is centrifuged and the supernatant liquid decanted into a clean tube. If the safranin has not been completely removed, the treatment is repeated with sufficient Lloyd's reagent to remove every trace of the dye.

Starting with diseased Turkish tobacco plants from the greenhouse, the supernatant liquid thus obtained is free of safranin, usually opalescent and amber or nearly colorless. Some preparations, however, have been obtained that, so far as the eye could detect, were water-clear.

Table 1 shows the infective power of various samples of juice and also the infective power of the purified-virus preparation obtained from the respective juice samples by the method described. The data in this table show that the infective power of the purified-virus solution was uniformly equal to, or generally exceeded, that of the juice from which it was prepared.

More recent results indicate that the decomposition of the safranin-virus precipitate is best accomplished from 20 per cent acetone. In experiment

TABLE 1.—Comparative infective power of the original juice and the purified virus preparation, obtained by precipitating the virus from the juice of diseased plants with safranin, then decomposing the precipitate with Lloyd's reagent

Exp.	Juice from diseased plants diluted with an equal volume of distilled water				rified viru com the c ice dilute volume	Check plants		
no.	Inocu	lated	Diseased	Inocu	Inoculated Dis		No.	No.
	No. of plants	No. of leaves	No. of plants	No. of plants	No. of leaves	No. of plants	used	diseased
1	100	1	14	100	1	56		
2	100	1	8	100	1	41		······································
3	100	1	14	100	1	27		
4	100	1	19	100	1	28		
5	100	1	21	200	1	49	100	1
6	100	1	21	100	1	29	100	1
7	100	1	15	100	1	42	100	2
8	100	1	11	100	1	24	100	1
9	100	1	26	100	1	28	100	0
10	100	1	9	100	1	35		
11	100	1	16	100	1	61	100	0
12	100	1	47	100	1	76	100	1

12 of table 1 the virus preparation was obtained by suspending the safranin-virus precipitate in 20 per cent acetone and decomposing with Lloyd's reagent. The dye seems to be more readily removed by Lloyd's reagent from 20 per cent acetone than from a purely aqueous suspension, and the infective power of the preparations containing 20 per cent acetone is fully as high as, if not higher than, the purely aqueous preparations. It is possible that the acetone decreases the basicity of the safranin and thus favors decomposition of the safranin-virus precipitate; or, it may decrease the basicity of Lloyd's reagent more than it does the safranin, thus making for a greater affinity for the safranin by Lloyd's reagent.

Use of Other Adsorbents. A number of other adsorbent earths have been tried for their pigment-removing power on juice from diseased tobacco plants and also on aqueous suspensions of the safranin-virus precipitate. Among the adsorbent earths, in addition to the Lloyd's reagent, that have been tried are: Fuller's earth, infusorial earth, kaolin, bentonite, and isarton. The German product, isarton, removes the safranin from an aqueous suspension of the safranin-virus precipitate more readily than does Lloyd's reagent, but at the reactions employed, isarton carried down the virus. Lloyd's reagent, however, removes the safranin from the safranin-virus precipitate in 20 per cent acetone more readily than does isarton. All

the other preparations were less effective in removing pigment from the juice and also from suspensions of the safranin-virus precipitate. Bentonite removed the virus from the juice of diseased plants at the reaction employed. Lloyd's alkaloidal reagent is outstanding in its property of removing pigment and leaving the virus in the supernatant liquid.

Effect of Suspended Material on Infectivity. Attention has already been directed to the fact that the purified virus preparations often show greater infective power than the juice from which they were prepared. Those purified preparations that show opalescence usually exhibit greater infectivity than preparations that appear clear to the eye.

Centrifuging at high speed for several minutes lowers the infective power of juice from diseased plants. It soon became evident that this lower infective power was probably not due to a greatly reduced virus content, since purified preparations from such juice were uniformly high in virus content, based on their infectivity.

These observations led to an investigation of the effect on infectivity of adding finely divided inert material to juice that had been centrifuged and also to purified virus preparations. When inert material was added to juice that had been centrifuged to form a fine suspension, the infectivity usually increased greatly.⁵ The data in table 2 show that finely divided charcoal is very effective in bringing about this increase in infective power. In experiments 2 and 6, table 2, the infectivity of the juice was not increased by the addition of activated charcoal. This may have been partly due to the short time of contact with the charcoal at relatively low temperature before inoculating plants.

It has been suggested that the charcoal removes a substance that inhibits the action of the virus. That this is not the only factor involved is evidenced by the fact that freshly expressed juice from diseased tobacco plants may be much more infectious before than after it is centrifuged at high speed. The suspended material must also play some other part in this phenomenon.

Virus preparations may, therefore, be obtained so nearly free of suspended material that their infectivity is low, but on adding finely divided material to form a fine suspension the infectivity may be restored. This is well illustrated in the case of juice of diseased plants in experiment 4, table 2. In this experiment the centrifuged juice, on being inoculated into plants, caused 25 plants, out of 100 inoculated, to become diseased. After the addition of activated charcoal to this same juice sample, 65 out of 100 plants inoculated, became diseased.

A still better illustration of the effect of suspended material on infectivity is given by the data in experiments 12 and 13, table 3. In experi-

⁵ Vinson and Petre, loc. cit., 1931 (footnote 2).

TABLE 2.—Influence on infective power of adding suspended material to a virus solution

Checks	No. of plants	Diseased	ja H i≥	6 1	Ħ	H	0		0	П
G	No. 0	$\mathbf{U}_{\mathbf{sed}}$	100	100	100	100	100		100	100
ared by decom- unin precipitate sponding juice) agent. Diluted lume of water	liseased out of inoculated	With activated charcoal	43 51	63 56	43			51	81	95
Virus solution pre posing the safi (from the corr with Lloyd's r	Virus solution prepared by decomposing the safranin precipitate (from the corresponding juice) with Lloyd's reagent. Diluted with an equal volume of water No. of plants diseased out of 100 plants inoculated		25 29	45	23 24			35	61	9.2
ed plants diluted olume of water	No. of plants diseased out of 100 plants inoculated	With activated charcoal	43	16	30	65	63	80	35	80
Juice from diseased plants diluted with an equal volume of water	No. of plants of 100 plants	Without activated charcoal	21	15	1	25	26	6	16	47
No. of leaves	No. of leaves inocu- lated per plant		T		⊢	-	H	H	. - 1	-
H.vnori.	ment no.		-	2	က	4	g	9	7	8

ment 12 the virus preparation caused 1 plant out of 100 plants inoculated, to become diseased; but, after the addition of activated charcoal, 31 out of 100 plants inoculated, became diseased. In experiment 13, inoculation with the virus preparation caused none of the 100 plants inoculated to become diseased; but, after the addition of activated charcoal, 32 out of 100 inoculated plants became diseased.

From these experiments it is evident that the infectivity of a given virus preparation may not be a satisfactory indication of the virus content. It is now considered necessary to inoculate plants, not only with the preparation but also with the preparation after the addition of a given quantity of activated charcoal, before a dependable idea as to the virus content can be obtained. After inoculating with a particular sample, 100 mgm. of activated charcoal were added to each 10 cc. of the sample in order to assure an excess of suspended material. With an excess of suspended material, it would seem that the latter is eliminated as a limiting factor in infectivity.

In order that relative infective powers of virus preparations be reliable indexes of virus content, the preparations should be comparable in amount and type of suspended material present.

Application to Enzymes in General. It should be emphasized here that the above-mentioned conditions may influence the activity of all enzymes, and, if this be true, the present data on enzyme purification may be quite misleading. For instance, it is easy to see how, with the presence of sufficient suspended material of the proper type, a small quantity of enzyme would show as high activity as the original starting material. Hence, with lowered total solids but unimpaired activity, a high degree of purification may have been deduced. Our experience with the virus of mosaic disease of tobacco shows how such results could be obtained without any purification whatever being effected.

Effect of Safranin Concentration on the Precipitation of the Virus. In precipitating with safranin the virus from juice of diseased plants, an excess of the safranin solution is required. This is shown by the fact that when precipitation is incomplete the solution is still highly colored with the dye. By further addition of safranin the precipitation of the virus may be carried to completion, or approximately so.

Diseased tobacco plants grown in the field have a higher virus content than those grown under conditions of reduced sunlight, as in the greenhouse. Juice from diseased field plants requires a higher concentration of safranin to completely precipitate the virus than does juice from greenhouse plants. This suggests that there may be a definite or stoichiometric relationship between dye and virus in the safranin-virus precipitate. With juice from diseased Turkish tobacco plants grown in the greenhouse, 5 cc.

TABLE 3.—Influence of safranin concentration on the precipitation of the virus from juice of diseased plants

Checks	No. of plants	Diseased									03	H		-	-	0	0	0	0	
Ch	No. of	$\mathbf{U}_{\mathbf{sed}}$. :								100	100		100	100	100	100	100	100	
removing pernatant cipitating ranin	No. of plants diseased	With activated charcoal									31	49	13	31	32	41			c 1	
Solution prepared by removing safrain from the supernatant liquid obtained on precipitating the virus with safranin	No. of plan	Without activated charcoal	9	J.	ന	0	9	0	0	0	24	23	ന		0	7	ന	0	-	
Solution safranin liquid ob	,	No. or plants in- oculated	10	10	10	10	10	10	10	10	100	100	100	100	100	100	100	100	100	
composing scipitate	No. of plants diseased	With activated charcoal									99	43	51	95	95					
Solution obtained by decomposing the safranin-virus precipitate		Without activated charcoal	6	10	10	∞	7	7	10	6	42	24	35	92	92	26	26	58	ı	
Solution o		No. of plants in- oculated	10	10	10	10	10	10	10	10	100	100	100	100	100	100	100	100		
No. of ce. of 1% aqueous	No. of cc. of 1% aqueous safranin solution added per 25 cc. of juice		20	10	15	20	2	10	15	20	ĭĊ	ıo	10	10	10	15	15	15	20	
No. of leaves in- oculated per plant			4	4	4	4	4	4	4	4	-	H	-	-	+		l 1		. 	
Exp.			la	2a	3a	4a	5b	Gb	71b	q8	q6						1.50		17a	

a Juice from plants grown in the field.

b Juice from plants grown in greenhouse, then placed in the open for a few weeks.

of a 1 per cent aqueous solution will bring about approximately complete precipitation of the virus from 25 cc. of the juice. For juice from diseased plants grown in the field, 2, 3, or even 4 times this concentration of safranin may be necessary to complete the precipitation of virus from 25 cc. of the juice.

Table 3 shows the effect of safranin concentration on the precipitation of the virus from juice of diseased Turkish tobacco plants. In experiments 5 to 16, inclusive, of table 3, the juice was obtained from potted diseased plants that had been grown in the greenhouse, then placed in the open for a few weeks. In experiments 1, 2, 3, 4, and 17, the juice was obtained from diseased field-grown plants. The data show that, with the addition of 5 cc. of 1 per cent safranin solution to 25 cc. of the juice, considerable virus remained in the supernatant liquid. In fact, the supernatant liquid, after removal of safranin, was, in the case of experiment 10, as infectious as the solution obtained by decomposing the safranin precipitate. With the addition of 15 cc. or more of the 1 per cent aqueous safranin solution, the infectivity of the supernatant liquid was greatly reduced.

Effect of Treating the Juice with Lloyd's Reagent Previous to Precipitating the Virus with Safranin. Lloyd's reagent, when added to juice from diseased plants, removes pigment but, as already mentioned, does not reduce the infectivity of the treated juice when not more than 1 gm. is used to 25 cc. Table 4 shows the relative infective power of virus preparations from the safranin precipitate when, in one case, the juice was treated with Lloyd's reagent before precipitating with safranin and, in the other case, when it was not so treated. The data show that in most instances preliminary treatment of the juice with Lloyd's reagent, prior to precipitating the virus with safranin, did not lower the infective power of the preparation over that obtained from juice that received no treatment with Lloyd's reagent.

In experiment 3, table 4, the difference in infective power of the 2 preparations could easily have been due to a difference in physical condition—one was clear and the other opalescent.

Treatment of the juice with Lloyd's reagent previous to the addition of safranin did not reduce appreciably the total solid or organic solid content of the virus preparation obtained on decomposing the safranin precipitate. This is well illustrated by the data of table 5. The total ash shown for the solutions in table 5 is derived mainly from the Lloyd's reagent. This must be so since the safranin precipitate contains very little ash and also since extracting Lloyd's reagent with a corresponding amount of redistilled water gave about the same amount of ash in the extract as shown in the solutions of table 5.

TABLE 4.—Effect of preliminary treatment of juice from diseased plants with Lloyd's reagent previous to precipitating the virus with safranin

Checks	No. of plants	Diseased				63		1	•
	No. 0	$\mathbf{U}_{\mathbf{sed}}$				100	100	100	100
Virus preparation obtained by decomposing the safranin precipitate from the juice with Lloyd's reagent. Juice not previously treated with Lloyd's reagent	No. of plants diseased out of 100 plants inoculated	With acti- vated charcoal				56	43		
Virus preparation obtained by composing the safranin prectate from the juice with Lloy reagent. Juice not previou treated with Lloyd's reagent	No. of plants 100 plants	Without activated charcoal	56	41	27b	42	24	28	28
Virus preparation obtained: juice first treated with Lloyd's re- agent, then the virus precipi- tated with safranin and the safranin-virus precipitate de- composed with Lloyd's reagent	No. of plants diseased out of 100 plants inoculated	With activated charcoal	1			63	43		
	No. of plants 100 plants	Without activated charcoal	53	32	11a	45	23	26	21
No. of leaves incen-	1		H	-	,	-	.		
Experi-	ment no.		1	2	က	4	5	9	7

a This preparation was water clear.
b This preparation was opalescent.

TABLE 5.—Effect of treating the juice with Lloyd's reagent, before adding safranin, on the solid and ash content of the virus preparation obtained by decomposing the safranin-virus precipitate with Lloyd's reagent

Sample number	diseased plants agent. The vir cipitated with	of juice from with Lloyd's re- us was then pre- safranin and the precipitate decom-	Virus preparation obtained by decomposing the safranin precipitate from 25 cc. of juice from diseased plants with Lloyd's reagent. Juice not previously treated with Lloyd's					
	Total solids in grams	Total ash in grams	Total solids in grams	Total ash in grams				
1	0.0124	0.0074	0.0134	0.0087				
2	0.0128	0.0084	0.0150	0.0085				
3	0.0138	0.0084	0.0133	0.0081				
4	0.0132	0.0085	0.0134	0.0081				
5	0.0132	0.0083	0.0135	0.0090				
6	0.0123	0.0081	0.0146	0.0089				
Total in 500 cc.	0.2590	0.1630	0.2773	0.1710				

Total solids in 500 cc. of the original juice—9.4260 gm.

DISCUSSION

The need for a simple and rapid method of securing purified virus preparations has been felt for some time, especially a method that is not time consuming and that will effect appreciable purification of the virus, giving highly active preparations that may be demonstrated to contain a majority of the virus of the starting material. The safranin procedure herein described is believed to meet the need mentioned. It is quite possible that the procedure will prove applicable to the purification of any substance that will give a precipitate with safranin. This procedure, if applicable to other viruses, will make it possible for biologists, in general, to obtain stable purified virus preparations, as no special technique and care are necessary. The method is almost proof against mistakes. For those biologists who are interested in and are actively carrying on cultural experiments with virus, this method should have a particular appeal, for, through its application, most of the substances accompanying the virus are eliminated and a stable preparation is obtained. Sterile juice from diseased plants is unstable, it clouds readily, and precipitates form on standing. The purified material is, therefore, much superior to the unpurified juice for purposes of inoculating media.

SUMMARY

Lloyd's alkaloidal reagent removes the safranin from an aqueous suspension of the safranin-virus precipitate, leaving the virus in the supernatant liquid.

The purified virus solutions thus obtained were fully as infectious as the juice of diseased plants from which they were prepared.

At the reactions employed, Lloyd's reagent has proved more satisfactory for decomposing the safranin-virus precipitate than kaolin, infusorial earth, Fuller's earth, bentonite, or isarton.

The addition of finely divided inert material to form a fine suspension often increases the infectivity of a virus preparation.

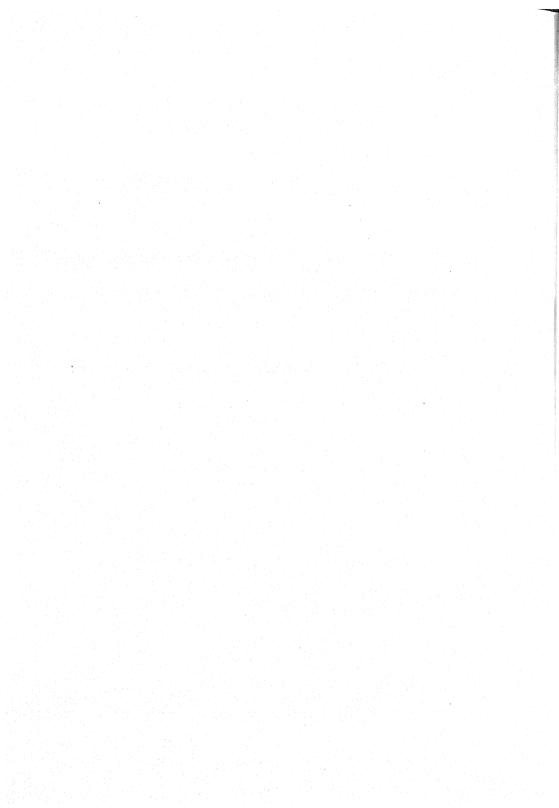
In order that relative infective powers of virus preparations be reliable indexes of virus content, the preparations should be comparable in amount and type of suspended material present.

The greater the virus concentration of a juice sample, the greater the amount of safranin required to bring about complete precipitation of the virus.

Previous treatment of the juice with Lloyd's reagent does not appreciably reduce the total solids in the preparation obtained by decomposing the safranin precipitate.

Total solids in the solution obtained by decomposing the safranin-virus precipitate with Lloyd's reagent represent about 3 to 4 per cent of the total solids of the original juice. About half of this 3 to 4 per cent, however, is ash that is derived mainly from the Lloyd's reagent.

University of Missouri, Columbia, Missouri.



PHYTOPATHOLOGICAL NOTES

Preliminary Note on a Phytophthora Root Disease of Chestnut.—A phycomycetous fungus that appears to be the same as Phytophthora (Blepharospora) cambivora (Petri) Buis., the cause of the ink disease of European chestnuts, has been isolated from the blackened roots of dying American chestnuts from Georgia, South Carolina, and Tennessee. The types of hyphae and sporangia and the dimensions of these, as well as most of the cultural characteristics, are similar to those ascribed to P. cambivora. To complete the identification, further studies are being made of the morphology and cultural reactions. Thus far the oospore stage, which is found in the cortex of infected seedlings in Europe, has not been found in our plants. Like European investigators of P. cambivora, we have had difficulty in isolating from naturally infected chestnuts this Phytophthora, which appeared in only 3 per cent of the total number of cultures, but it was easily isolated from young plants from known infested areas. Reisolations from dead inoculated plants were readily made.

Four months after inoculations in the greenhouse with the American Phytophthora, there had died 72 per cent of 82 seedlings of the native Castanea dentata (Marsh.) Borkh., 74 per cent of 43 seedlings and 17 per cent of 54 larger plants of the European C. sativa Mill., 67 per cent of 32 seedlings of southern chinquapins, 3 per cent of 63 seedlings and 5 per cent of 57 larger plants of the Japanese C. crenata Sieb. and Zucc., and none of 14 seedlings and 2 per cent of 56 larger plants of the Chinese C. mollissima These percentages are not strictly comparable, because all the inoculations were not made simultaneously, because some of the inoculations were made in wounds and others in soil and because the plants varied in age and were from 1 to 4½ feet high. Some of the plants are still dying. The native C. pumila (L.) Mill. is also susceptible to the organism, but, so far, neither the Chinese C. henryi Rehd. and Wils. nor the Chinese C. seguinii Dode has become infected. Two of 81 inoculated seedlings of Quercus prinus L. have also become infected. In an experimental planting of young chestnut plants in infested soil in Georgia, 3 plants out of 6 of C. sativa died in the first season and 39 plants of C. mollissima and 25 of C. crenata appeared healthy. In the examinations of many thousands of plants in the experimental forest plantings of C. crenata and C. mollissima in all of the Southern States no case of the root disease has been noted. The high susceptibility of the American and European chestnuts and the comparative resistance of Asiatic chestnuts to the American Phytophthora agree with the results of European investigators of P. cambivora.

The facts that a few weeks' search in the South was sufficient to establish the presence of the American Phytophthora in 3 States, that the dving of native growth from blight (Endothia parasitica (Murr.) P. J. and H. W. Anderson) makes the recognition of trees dying from other causes difficult in many places, and that it is difficult to culture the fungus from naturally infected roots indicate that the disease is widespread in the South and suggest the possibility that the fungus may have been present in this country for many years. Dying and recession of the chestnut at the lower altitudes at various places from Maryland to Mississippi have been occurring for about 100 years. These have been attributed to the Armillaria root rot, to forest fires, and to other causes, without an exhaustive study having been made. It is strongly suspected that this Phytophthora root disease has played an important, if not the primary, part in this recession. If subsequent study verifies the identity of the American Phytophthora and Phytophthora cambivora of Europe, the reason for the minor differences observed may be that these strains may have grown for many generations on different hosts and on two continents. The comparative resistance of the four species of Asiatic chestnut and the marked susceptibility of American and European species make one suspect that the original home of this fungus, as of the chestnut blight, is Asia.

The symptoms of this disease varied considerably on the trees examined. Some large trees wilted suddenly and others gradually died from the top downward over a period of 2 years or more. Frequently a surface root remained alive for some time after the other roots were killed. Taproots of most of the trees were more severely affected than lateral ones, and from many of the larger roots an ink-like slime oozed from the affected areas and discolored the attached soil. In some cases the roots were blackened where they joined the crown but were normal in their outer parts. In other cases isolated cankers occurred on the roots.

Pathologists and others interested are requested to report cases where the presence of this disease is suspected.—Margaret Milburn and G. F. Gravatt, Division of Forest Pathology, Bureau of Plant Industry, United States Department of Agriculture, Washington, D. C.

The Odor of Bunt Spores.—In a recent paper an account was given of the isolation of trimethylamine from spores of Tilletia levis Kühn collected at Winnipeg, Canada, in 1931. This substance was considered to be responsible for the odor usually associated with bunt spores. Spores of T. tritici (Bjerk.) Wint., also grown at Winnipeg, in 1931, did not possess this odor and trimethylamine could not be isolated from them.

¹ Hanna, W. F., H. B. Vickery, and G. W. Pucher. The isolation of trimethylamine from spores of *Tilletia levis*, the stinking smut of wheat. Jour. Biol. Chem. 97: 351-358. 1932.

In the discussion of these results the writers made the following statement: "It has been shown by several workers that *Tilletia tritici* comprises several physiologic forms which differ from one another in their parasitic capabilities. The possibility exists that certain of these forms may be able to produce trimethylamine, whereas others may not. The spores of *Tilletia tritici* used in this investigation were considered to belong to a single physiologic form. However, if the hypothesis just suggested is correct, other forms of this fungus may occur, the spores of which contain trimethylamine."

The writer of this note has since had opportunity to examine spores of *Tilletia tritici* at the Station Centrale de Pathologie Végétale, Versailles, France. These spores gave off a faint odor of trimethylamine. Spores of several physiologic forms of *T. tritici* kindly supplied by Dr. E. F. Gaines, of the State College of Washington, Pullman, Washington, have also been examined and they, too, smelled distinctly of trimethylamine. In view of these observations it is evident that trimethylamine may be present in the spores of some strains of *T. tritici* and absent from those of others.

Using pure cultures made from single secondary conidia, which are apparently haploid, crosses have been made between *Tilletia levis* and the strain of *T. tritici* lacking trimethylamine. The first-generation hybrid spores resulting from this cross resemble in appearance those of the *T. levis* parent, as has already been reported by Flor,² and emit an odor of trimethylamine. In this cross, therefore, the factors for smooth spore wall and odor are dominant.—W. F. Hanna, *Dominion Rust Research Laboratory*, Winnipeg, Canada.

New Developments in treating Pecan Rosette with Chemicals. Experiments conducted in the latter part of the growing season of 1931 indicated that pecan rosette could be prevented by treating the leaves with an 0.8 to 1.0 per cent solution of ferric chloride or ferric sulphate, as reported in a recent issue of Phytopathology.¹

With the enlargement of these experiments undertaken at the beginning of the 1932 season, more or less conflicting results began to appear. The experiments included injection of iron salts into dormant trees; application of iron salts to the soil around the trees, while they were still dormant; after the foliage was well developed, spraying by various procedures with both ferric and ferrous sulphate; and dipping terminal branches that showed the rosette symptoms in solutions of iron salts. The only favorable results were

² Flor, H. H. Heterothallism and hybridization in *Tilletia tritici* and *T. levis*. Jour. Agr. Res. 44: 49-58. 1932.

¹ Alben, A. O., J. R. Cole, and R. D. Lewis. Chemical treatment of pecan rosette. Phytopath. 22: 595-601. 1932.

associated with the small-scale experiments where galvanized iron containers were used in handling the solutions. It was further found that the iron salts used contained appreciable quantities of zinc. Accordingly, a small series of experiments were undertaken, using shellacked crocks instead of galvanized buckets. The effects of zinc sulphate and zinc chloride solutions were compared with the effect resulting from iron sulphate solution. One immersion in the iron solution failed visibly to improve conditions, while zinc sulphate and zinc chloride solutions restored the new leaves back to normal condition. These results were secured with trees located on both alkaline and acid soils.

Roberts and Pierce² reported beneficial effects on peach foliage following zinc-lime sprays used for controlling bacteriosis, and, accordingly, in our further experiments, zinc sulphate and zinc-lime sprays were included. Trees sprayed with zinc lime 2–2–50 were greatly improved but not so much as were those trees sprayed with zinc sulphate. The most satisfactory results were obtained by using an 0.18 per cent zinc sulphate (ZnSO₄·7H₂O) and 0.012 per cent zinc chloride (ZnCl₂) solutions. Burning of the foliage resulted when stronger solutions were used. The chlorine radical is apparently more toxic than the sulphate radical; at least 7.1 times more zinc was applied without burning with the sulphate form than with the chloride.

These experiments were practically completed before our attention was directed to the experiments of Chandler, Hoagland, and Hibbard,3 reporting on little leaf or rosette in fruit trees and noting iron sulphate treatments of apple, pear, peach, plum, cherry, and walnut, as well as grape. They found that iron applications to the soil gave generally favorable but frequently erratic results and report that zinc, rather than the iron, itself, is the corrective factor. While not definitely excluding the possibility of zinc as an essential element for the fruit trees, their conclusions emphasize the probable changes in soil constitution and soil flora, and experiments with injections of zinc solutions and spraying are suggested as desirable for clarification of the problem. They report that, although the iron solutions when free of zinc impurities appeared without effect, larger quantities of pure zinc salts are required to secure the favorable responses of the trees than when mixed solutions of iron and zinc are employed, which, apparently, explains our favorable results with iron solutions containing quantities of zinc somewhat lower than those reported above as the optimum for the zinc solutions.

² ROBERTS, JOHN W., and LESLIE PIERCE. Zinc-lime: a fungicide for the peach. Phytopath. 22: 415-427. 1932.

³ CHANDLER, WM. H., D. R. HOAGLAND, and P. L. HIBBARD. Little-leaf or rosette, in fruit trees. Proc. Amer. Soc. Hort. Sci. 28 (1931): 556-560. 1932.

Our experiments in spraying foliage with zinc sulphate or zinc-lime sprays and also the experiments in dipping terminal parts of branches showing pronounced rosette symptoms in zinc solutions and the prompt and favorable response of the trees to such treatments apparently indicate that zinc is an essential element for the healthy development of the pecan tree.—A. O. Alben, Bureau of Chemistry and Soils; J. R. Cole, Bureau of Plant Industry; and R. D. Lewis, Bureau of Chemistry and Soils, U. S. Department of Agriculture, Shreveport, Louisiana.



REPORT OF THE FIFTH ANNUAL COTTON-ROOT-ROT CONFERENCE¹

The fifth annual conference of workers with the cotton-root-rot disease caused by *Phymatotrichum omnivorum* (Shear) Duggar was held at the University of Texas, Austin, Texas, on February 1 and 2, 1932. The meeting was attended by 33 plant pathologists, agronomists, soils specialists, chemists, botanists, and horticulturists connected with the work, and approximately 50 visitors.

Director A. B. Conner of the Texas Agricultural Experiment Station and Dr. J. J. Skinner, Bureau of Chemistry and Soils, U. S. Department of Agriculture, presided over the various sessions. Following an address by Director Conner on the purpose of the conference, the 55 technical reports of experimental work were presented during the first day; these are summarized below in their order on the program. On the second day, general summaries of the progress of the work were presented in 3 addresses, one by Dr. J. J. Taubenhaus of the Texas Agricultural Experiment Station; another by Dr. D. C. Neal of the Bureau of Plant Industry, U. S. Department of Agriculture; and another by Mr. Paul R. Dawson, of the Bureau of Chemistry and Soils, U. S. Department of Agriculture.

STUDIES ON LIFE HISTORY

PHYSIOLOGIC SPECIALIZATION OF Phymatotrichum omnivorum

Morphological behavior of different isolations. During 1931, 2 additional strains of the fungus, after 14 to 18 months' growth on artificial media, lost their capacity to produce strand hyphae or sclerotia. In several isolations of the fungus, specialized fusion of the large-cell hyphae has been observed, the appearance being similar to that of conjugation of certain filamentous algae. (D. C. Neal and K. C. Gunn, Bureau of Plant Industry, U. S. Department of Agriculture).

Inoculation experiment with physiologic forms. Nine different isolations, of which some belonged to each of 5 physiologic forms (cultural) of the fungus, caused typical root rot of inoculated cotton plants, irrespective of the sources from which the cultures had originally been isolated (cotton, beets, carrots, and alfalfa) and irrespective also of the length of time the cultures had been grown on artificial media (3 months for the youngest to over 3 years for the oldest strain). Three strains that had grown the more slowly in artificial culture also caused the slower development of root rot. (W. N. Ezekiel and J. J. Taubenhaus, Texas Agricultural Experiment Station).

¹ Prepared for the conference by the following committee: W. N. Ezekiel, Chairman, D. C. Neal, Paul R. Dawson, and E. B. Reynolds.

GROWTH AND NUTRITION OF THE FUNGUS

Nutritional studies on growth in artificial culture. The growth rate of the fungus was not affected by the relative concentration of dextrose in culture solutions until the supply was almost exhausted. Preliminary tests gave no evidence of production of staling products during the period of active growth. Carrot juice added to synthetic media resulted in marked increase in growth; an accessory growth-promoting substance may be involved, but certain results suggest that the effect may be due merely to the additional sources of nitrogen. (W. N. Ezekiel, J. J. Taubenhaus, and J. F. Fudge, Texas Agricultural Experiment Station).

Growth in synthetic media. Ammonium nitrate and ammonium sulphate, each used in Duggar's solution at rates to supply about 12.4 gm. of nitrogen per liter, inhibited growth of the fungus; while calcium nitrate, sodium nitrate, and potassium nitrate at the same rates yielded abundant growth, the best being with the calcium nitrate. The filtrates of these cultures became less acid as growth of the fungus progressed. (D. C. Neal, R. E. Wester, and K. C. Gunn, Bureau of Plant Industry, U. S. Department of Agriculture).

Effects of anaerobic conditions on growth. Growth was inhibited by anaerobic conditions and notably restricted by carbon dioxide concentrations above 25 per cent. The fungus did not grow with 100 per cent of nitrogen or carbon dioxide, but after such exposure for 8 days was still able to grow when returned to aerobic conditions. Aerobic requirements for growth of the fungus may explain effects on root rot following subsoiling. Such treatment may activate the sclerotia, thereby lessening the chances of survival of the fungus through the winter. (D. C. Neal and R. E. Wester).

Greenhouse studies of Phymatotrichum omnivorum. Attempts to inhibit growth of the fungus or control infection by competition of other soil organisms met with failure. Cultures of 2 species of Fusarium, 2 of Aspergillus, 1 Mucor, and 1 Penicillium introduced into soil cultures did not prevent root-rot infection of cotton plants following inoculation from cultures of P. omnivorum. (D. C. Neal and L. G. McLean, Bureau of Plant Industry, U. S. Department of Agriculture).

Preliminary irradiation studies. Six-day and 2-week old cultures, respectively, were irradiated for 2 to 30 minutes under a mercury-vapor lamp, with the view of inducing sporulation. With the longer irradiation, definitely injurious results were noted, but no sporulation was observed. (J. J. Taubenhaus and W. N. Ezekiel).

SCLEROTIAL STAGE OF THE FUNGUS

Distribution of sclerotia in infested areas. In an alfalfa field in Arizona sclerotia were most abundant at 6 to 24 in. deep, with a few at a depth of

54 in. Near a dead Chinese elm tree, 8,203 sclerotia were obtained, most abundantly at 6 to 42 in., but a few at a depth of 84 to 90 in. Sclerotia were distributed unevenly in the soil, occurring in pockets. (C. J. King, Bureau of Plant Industry, U. S. Department of Agriculture).

Occurrence of sclerotia with regard to soil types and hosts. A post-hole digger was used to sample soil to depths of 2 ft. for sclerotia, which were recovered by washing soil through sieves with running water. Sclerotia were found in 13 counties in Texas, in soil of 14 different types, near 27 different affected hosts. (J. J. Taubenhaus and W. N. Ezekiel).

Occurrence of sclerotia in nature. In excavations in Wilson soil at the Greenville Station, sclerotia were found near old tree roots and in primary centers of infection in fields where cotton followed several years of clean fallow or nonsusceptible crops rather than in continuous cotton plots. They were found usually in groups, at depths of 4 to 18 in., but mostly at 4 to 12 in., and near small cotton rootlets rather than along the taproots of the plants. (H. C. McNamara and D. R. Hooton, Bureau of Plant Industry, U. S. Department of Agriculture).

Observations on the formation of sclerotia in culture. Growth of the fungus in films of media, inverted over special paraffin-beeswax cells, was followed microscopically. The hyphae anastomosed and entangled to form pseudosclerotia. These differed microchemically and morphologically from sclerotia, as produced in soil or cultures, particularly by the absence of the cuticularized outer wall of the sclerotia. (Elizabeth Moore, University of Texas).

Factors affecting sclerotia production. Formation of sclerotia in synthetic media of many formulas correlated closely with nutritive conditions suitable for rapid and abundant vegetative growth of the fungus and was inhibited by any changes detrimental to growth, suggesting that field treatment militating against free growth of the fungus might have a further desirable effect in discouraging production of sclerotia. (W. N. Ezekiel, J. J. Taubenhaus, and J. F. Fudge).

Laboratory studies on the longevity of sclerotia. Sclerotia secured in the summer of 1929 from a carrot field at Temple were stored in small vials filled with moist Houston clay-loam soil. After more than 2 years, germination tests showed many of these sclerotia still viable and capable of vigorous growth. (J. J. Taubenhaus and W. N. Ezekiel).

SPORE STAGES OF THE FUNGUS

Inoculations with Phymatotrichum spores. Cotton plants grown in containers at College Station were inoculated periodically with Phymatotrichum spores, of which some were placed near the uninjured taproots, others introduced through epidermal or cambial injuries, and others in-

jected into the cambium of the root. No infection occurred, although high percentages of infection were secured with check plants inoculated with fresh cotton-root inoculum. (J. J. Taubenhaus and W. N. Ezekiel).

HOST PLANTS

New hosts, symptoms. New additions to the root-rot host list from the Temple substation included 18 species of weeds and 27 species and varieties of ornamentals. (S. E. Wolff, Texas Agricultural Experiment Station).

Inoculation experiments with cacti. Ornamental cacti of a number of genera and species succumbed to artificial inoculation with root rot, while noninoculated plants remained normal. (J. J. Taubenhaus and W. N. Ezekiel).

Further inoculation experiments with monocotyledons. No infection was secured at College Station in continued inoculations during 1931 on monocotyledonous plants, including corn, sorghum, and various liliaceous plants; although high percentages of infection occurred on the interplanted cotton and carrot plants used as inoculated checks. Monocotyledonous plants are apparently immune from Phymatotrichum root rot. (J. J. Taubenhaus and W. N. Ezekiel).

INFECTION AND RESISTANCE

Histologic studies on the process of infection. Cotton seedlings, grown aseptically in tubes of agar, and inoculated with sclerotia from a pure culture of Phymatotrichum omnivorum, were fixed at intervals. After 5 days, the fungus was present in the first outer layer of epidermal cells; after 8 days it had penetrated the parenchyma; and after 12 days, had entered the vascular system and pith of affected rootlets. Invasion was both inter- and intracellular. (J. J. Taubenhaus and W. N. Ezekiel).

Reaction of cotton seedlings grown in the presence of pure cultures of Phymatotrichum omnivorum. Pea, bean, and cotton seedlings grown in soil cultures of the fungus in the laboratory failed to become infected, although sclerotial masses in the cultures were penetrated by the roots of the plants. (L. G. McLean).

Growth of the fungus in plant juices as correlated with resistance of plants to root rot. Phymatotrichum omnivorum grew profusely in undiluted, expressed juices from dicotyledonous plants, susceptible to root rot, but was generally inhibited in undiluted juices from roots of monocots, which are immune. Good growth occurred, however, in diluted juices from the monocots, indicating that immunity is due probably to specific toxic materials in monocots. (W. N. Ezekiel, J. J. Taubenhaus, and J. F. Fudge).

HIBERNATION AND TRANSMISSION OF ROOT ROT

Rôle of winter and spring vegetation at Temple. A study of natural infection of 3 winter annuals at Temple, Texas, showed no infection until about April 20. With Sitilias multicaulis, 9 per cent of the plants were infected by April 27 and 58 per cent by June 1, when only 13 per cent of Hamosa nuttalliana plants were infected and none of Vicia leavenworthii. Under the same conditions, 25 per cent of the perennial Ipomoea trifida plants were infected by February 9, and 53 per cent by May 15. (S. E. Wolff).

Rôle of winter and spring vegetation in south Texas. In fields in Bexar and Dewitt Counties, grown continuously in cotton, in corn following cotton, and in fallow following cotton, respectively, 14 species of winter and spring weeds assisted in hibernation of Phymatotrichum. In February, 1930 and 1931, 1 to 5 per cent of these weeds showed root rot, and by May, infection ranged from 1 to 15 per cent. (J. J. Taubenhaus and W. N. Ezekiel).

Nursery plants as possible carriers. Balled and nonballed nursery plants from a root-rot-infested area were transplanted into root-rot-free soil at College Station. Some of the nursery plants, as well as interplanted cotton plants, succumbed to typical Phymatotrichum root rot, presumably transmitted on infected roots of the nursery plants or as sclerotia in the balled soil. Check plants remained free of root rot. (J. J. Taubenhaus and W. N. Ezekiel).

OCCURRENCE OF ROOT ROT AND ESTIMATION OF LOSSES

Possible occurrence in other countries. A puzzling cotton disease in India had been considered possibly Phymatotrichum root rot. Microscopic examination of infected cotton roots from India and cultures from the roots indicated that the disease is distinct from Phymatotrichum root rot. The symptoms resembled alkali injury as commonly found in El Paso County. Sclerotium bataticola was usually present. (J. J. Taubenhaus and W. N. Ezekiel).

The root-rot fungus indigenous to Arizona deserts. Conidial mats of the fungus were found along a roadway, in a desert area 12 miles from the nearest cultivation. Mycelium of the fungus was found on the roots of 6 native plant species, and sclerotia were found in the soil. (C. J. King).

Root rot in river bottoms. Typical Phymatotrichum root rot has recently been found in flooded river bottoms, along the Trinity River and in a few places along the Brazos River, on roots of cotton plants and of native pecan stumps that were still alive and sprouting in spite of continued cultivation. (J. J. Taubenhaus and W. N. Ezekiel).

Occurrence of root rot in certain alluvial soils. Although root rot did not occur in the major portions of the alluvial soil areas in Bell and Milam Counties, Texas, in 1931, the disease was prevalent in certain individual fields on Catalpa, Ochlockonee, and Trinity clay soils. (H. E. Rea, Texas Agricultural Experiment Station).

Root rot in relation to soil types and moisture. At the Temple substation, root-rot infection for 4 years averaged 39.5 per cent on a Houston clay area, in which the percentage of soil moisture averaged 19.68 per cent in the first 3 ft.; while infection was 56.2 per cent on a deep phase of Houston black clay in which moisture percentage averaged 29.47 per cent. Wilting point on the Houston clay is about 12 per cent and on the Houston black clay about 19 per cent. (Henry Dunlavy, Texas Agricultural Experiment Station).

Root-rot losses in Texas. Estimates for root-rot losses on cotton, as prepared by the Division of Crop and Livestock Estimates, were discussed. For the few years for which data were available, there was an apparent high correlation between root-rot and boll-weevil damage. (F. H. Whitaker, Bureau of Agricultural Economics, U. S. Department of Agriculture).

Estimation of cotton-crop losses. Yields of cotton in a root-rot area were corrected for seasonal weather influences by comparison with yields in a near-by bottomland field where root rot did not occur. For the 6-year period, the estimated percentage of the crop lost from root rot in one continuous cotton plot averaged about 69 per cent of the percentage of plants killed by the disease and for another plot, about 50 per cent. It is suggested that percentage reductions in yield may be estimated roughly as averaging about 50 per cent of the percentages of plants killed by root rot prior to the first picking. (W. N. Ezekiel and J. J. Taubenhaus).

Date of wilting as related to losses. In some experimental plots at College Station, cotton plants killed by root rot 2 months or more before first picking bore practically no crop; plants killed a little more than a month before harvest averaged less than a boll and only 0.79 gm. of lint per plant; plants succumbing 3 weeks before harvest averaged more than 2 bolls and 3.21 gm. of lint per plant—as compared to 2.3 bolls and 4.6 gm. of lint average for normal plants. (W. N. Ezekiel and J. J. Taubenhaus).

CONTROL STUDIES

Soil Conditions, Including Soil Reaction

Root-rot occurrence in relation to chemical characteristics of soils. While root-rot infestation is, in general, heaviest on soils of calcareous nature and alkaline reaction and is lightest on noncalcareous soils of neutral or slightly acid reaction, data do not indicate a definite correlation with the content of calcium carbonate or the pH, particularly within a

given soil type of Texas blackland soils. On the other hand, there appears to be, as a rule, a significantly higher content of organic carbon and total nitrogen, as well as a higher carbon-nitrogen ratio, in soils from noninfested areas. Similarly, examination of soils from localized root-rot spots reveals a marked trend toward a lower content of reactive or available organic matter than is the case with soils from root-rot-free areas of the same fields. In a Wilson clay-loam field, where the carry-over and initial appearance of root rot each season are largely confined to limited areas of shallow-phase soil, the less infested deep-phase soil is distinguished by a lower calcium carbonate content, a lower pH, a higher available moisture and nitrate content throughout the crop season, and by a higher organic carbon content, total nitrogen, carbon-nitrogen ratio, and available phosphoric acid. In the blackland soils studied, the chemical differences most consistently correlated with relative or complete freedom from root rot are those indicative of a lower degree of erosion damage and a higher state of fertility. (E. R. Collins, W. V. Black, D. R. Ergle, and P. R. Dawson, Bureau of Chemistry and Soils, U. S. Department of Agriculture).

Studies on basis of the soil-reaction correlation with root rot. In originally acid soils, in which various salts were incorporated, growth of the fungus in soil chambers was not increased with additional available supplies of calcium, sulphate, phosphate, nitrate, and probably potassium ions beyond that expected from the relative changes in pH. The favorable effect of alkaline soils is probably not the specific effect of any of these ions. (W. N. Ezekiel, J. J. Taubenhaus, and J. F. Fudge).

A field-plot test of sulphur for the gradual control of root rot. Applications in 1929, to plots of noncalcareous Lufkin soil at College Station, of sulphur at low rates based on laboratory tests, resulted in progressive decrease of root rot in the 3 treated plots; while the disease has become more prevalent in 3 lime-treated plots and varied individually in the 6 check plots. (W. N. Ezekiel, J. J. Taubenhaus, and J. F. Fudge).

FERTILIZER APPLICATIONS

A 12-year manurial experiment on root-rot control. Application of organic manures in deep furrows beneath cotton rows was effective in reducing infestation in an experiment at Sacaton, Arizona, conducted for 12 years. Corral manure, at 12 tons per acre for 8 years, reduced root rot from 72 per cent to 1.1 per cent; there was a general decrease of infestation also in the control plots, but not to the same extent. (C. J. King, Claude Hope, and E. D. Eaton, Bureau of Plant Industry, U. S. Department of Agriculture).

Effects of fertilizer in relation to root-rot control. In the 1931 experiments in the blackland region of Texas by the Bureau of Chemistry and

Soils, as in previous years, properly proportioned ratios of nitrogen and phosphoric acid accelerated maturity and increased yields to such a degree as to be effective in completely offsetting root-rot losses. On cotton following sorghum, nitrogen fertilizers offset the depressing effect of the latter, which is one of the most valuable rotation crops for root-rot-infested ground. Experiments repeated during 3 to 4 years on the same plots showed that the benefits of fertilizers are cumulative, due to residual effects. Fertilizers most effective in accelerating plant growth and increasing cotton production caused actual reduction in the proportion of plants dying from the disease. This direct effect of fertilizers on root rot assumes significance only where the severity of infestation has first been reduced by rotation or tillage treatment or after several successive years of fertilizer application. (H. V. Jordan, J. H. Hunter, and P. R. Dawson, Bureau of Chemistry and Soils, U. S. Department of Agriculture).

Response of cotton to commercial fertilizers under root-rot conditions. In cooperative experiments in Bell County, use of 4-8-4, 4-8-0, and 4-0-0 fertilizers at 400 lbs. per acre increased yields 132, 145, and 48 lbs. of seed cotton per acre, respectively, in 15 tests on Houston black clay; 83, 84, and 35 lbs. in 9 tests on Houston clay; and 111, 99, and 51 lbs. in 9 tests on San Saba clay. (H. E. Rea).

Concentration of soluble salts as affecting growth of the fungus in soils. In soil-chamber tests with 3 soil types, growth of the fungus was prevented with around 5 per cent concentration in the soil solution of soluble salts, such as potassium nitrate, ammonium nitrate, sodium chloride, and potassium phosphate. Insoluble materials, such as calcium carbonate and calcium phosphate, apparently had no toxic effects, even at higher concentrations. (W. N. Ezekiel, J. J. Taubenhaus, and J. F. Fudge).

SOIL DISINFECTANTS

Treatment of cotton root rot with ammonia. In cultures, both mycelia and sclerotia were killed by dilute ammonium hydroxide and by short exposures to the gas. Forty hours after applying 6 per cent ammonium hydroxide solutions around large cotton plants in the field, the plants were removed and placed in moist chambers. No mycelial growth developed on the treated roots. (D. C. Neal).

Treatment of primary centers of infection with formalin. Applications of various chemicals at Greenville for 3 seasons to primary centers of infection suggest the feasibility of such control for small areas. In 1931, 2 per cent and 4 per cent formaldehyde solutions were applied in Wilson clay soil and penetrated 12 to 18 in. within 24 hours. A majority of the centers treated early in the season showed no further advance of the disease, although late-season treatments were not so effective. (H. C. McNamara).

Field-plot tests with some soil disinfectants. Disinfectants have been incorporated yearly since 1929 in field plots, at College Station, planted to cotton. During 1931, good control was obtained in plots treated with some of the chlorophenol-mercury and nitrophenol-mercury compounds, while root rot caused almost total loss in the check plots. (J. J. Taubenhaus and W. N. Ezekiel).

Laboratory tests of initial and residual efficiency of disinfectants. In soil-chamber tests, 3 organic-mercury compounds of the ethyl-mercury type showed as high initial efficiency as chlorophenol-mercury and nitrophenol-mercury compounds of much higher mercury contents. After contact with the soil for a month, however, the fungicidal value of the ethyl-mercury products had decreased, while that of the other materials was approximately unchanged. Phenyl mercury acetate proved relatively inefficient. (W. N. Ezekiel and J. J. Taubenhaus).

Sodium chloride, gasoline, and some other disinfectants. Under field conditions, at College Station, sodium chloride at the rate of 3,000 lb. per acre failed to control root rot, which is in agreement with results of laboratory tests, which suggest that concentrations required would approximate 20,000 lb. of sodium chloride per acre. (J. J. Taubenhaus and W. N. Ezekiel).

BARRIERS TO LIMIT SPREAD OF ROOT ROT

Sulphur barriers and graminaceous crop barriers. Barriers of soil containing 2 or 4 per cent of sulphur and barrier rows of sorghum were again tested both in boxes and under field conditions at College Station. In no case did root rot spread from the inoculated cotton plants on one side of the various barriers to the noninoculated plants on the other side. (J. J. Taubenhaus and W. N. Ezekiel).

Root-rot barriers in the field. Root rot has not crossed earth barriers containing crude carbolic acid, waste motor oil, or open trench barriers 2 ft. deep, in tests for 4 years at Greenville. In 1931, no spread was observed through barriers of manure, lignite, sulphur, calcium chlorate, and waste oil, while spread was observed beyond salt, marcasite, and sand barriers. (H. C. McNamara and D. R. Hooton).

SUBSOILING

Effects of subsoiling on occurrence and spread. Portions of 6 heavily infested fields on Houston soils were thoroughly subsoiled during the summer and early fall of 1930. In 1931, the numbers of cotton plants killed by root rot in the subsoiled areas were reduced in all cases (to 5 to 9 per cent) until after the crop was practically mature, while on the nonsubsoiled areas the losses remained very heavy (40 to 70 per cent). This effect was associated with a lower rate of spread of root rot from early centers of

infection on the subsoiled plots. Increases in yield from reduction in root rot and increased productivity as a result of subsoiling ranged from nearly 300 lb. to over 800 lb. of seed cotton per acre. The tillage was equally effective, whether it followed a rotation crop of sorghum or a highly infected cotton crop. During the early growing season the subsoiled plots showed a significantly higher total soil-moisture content and a distinct trend toward a higher available moisture, higher nitrate, and higher available organic-nitrogen content. There is evidence that factors such as these, favoring plant growth and productivity on subsoiled ground, play a rôle in the suppression or delay in spread of root rot. (P. R. Dawson, H. V. Jordan, and E. R. Collins).

Effects of deep tillage on root rot. In November, 1930, 15 plots at the Temple substation were subsoiled to a depth of 15 to 18 in. In 1931, 11 of the subsoiled plots had less root-rot infection at the end of the season than adjoining check plots, and 12 made a higher yield. Subsoiled plots averaged 32 per cent root rot and yielded 735 lb. of seed cotton per acre, while adjoining checks averaged 46 per cent root rot and yielded 573 lb. of seed cotton. (H. Dunlavy).

ROTATION

Root rot in rotation. An acre of cotton at the Temple substation, which showed over 99 per cent root-rot infection in 1927, was planted to nonsusceptible crops for the next 3 years, replanted to cotton in 1931, and the root-rot infection was 13.7 per cent at the end of the season. An adjoining acre, also with 99 per cent infection in 1927, was planted continuously to cotton and developed 41 per cent infection in 1931. (H. Dunlavy).

Root rot in various rotation and cultural treatments. In experiments at the San Antonio Field Station for 23 years, root-rot losses have been less with cotton grown in rotation with nonsusceptible crops, such as corn, sorghums, or small grains, than with cotton grown continuously on the same land. Three-year rotations are little, if any, less effective than 4-year rotations but are appreciably more effective than 2-year rotations. Persistent carry-over in plots in rotations was definitely associated with survival of the fungus on dead roots of native trees. (Geo. T. Ratliffe, Bureau of Plant Industry, U. S. Department of Agriculture).

Results of a 42-month fallow. Viable sclerotia were found to a depth of 17 in. under wilting cotton plants, planted at Temple in plots following 42 months of clean fallow. Large numbers of nonviable sclerotia were found 21 and 28 in. deep. (S. E. Wolff).

The effect of rotation on root rot. In rotations at Weslaco, initiated in 1927, plots planted to sorghum or corn in 1930 showed no root rot in the cotton at harvesting time in 1931. Other plots in which susceptible crops

had been grown in 1930 showed 68 to 100 per cent root rot. Yields of cotton following sorghum or corn were more than twice those obtained with cotton following cotton. (W. J. Bach, Texas Agricultural Experiment Station).

RESISTANT SPECIES, VARIETIES, AND STRAINS

Grape varieties. At Weslaco, varieties of grapes previously reported promising as resistant rootstocks again showed resistance in further tests, which included artificial inoculation of the plants. (W. J. Bach and J. J. Taubenhaus).

Nursery and ornamental plants. The hackberry, live oak, and yaupon develop complete resistance as the plants become older. The coralberry was found to be resistant to root rot in tests at College Station. (J. J. Taubenhaus and W. N. Ezekiel).

Nursery plants. Species of the mulberry and willow families were found very susceptible, while of 472 individuals in 11 species of the peach family tested at Temple only 2 plants died. Members of the honeysuckle family showed high resistance. (S. E. Wolff).

Alfalfa varieties and other legumes. Of a large number of alfalfa varieties and other legumes that were tested at Iowa Park substation for possible resistance none has yet shown any promise of even partial resistance to root rot. (J. J. Taubenhaus and L. E. Brooks).



REPORT OF THE SIXTEENTH ANNUAL MEETING OF THE PACIFIC DIVISION OF THE AMERICAN PHYTOPATHOLOGICAL SOCIETY

The meetings of the Pacific Division of The American Phytopathological Society were held in conjunction with those of the Pacific Division of the American Association for the Advancement of Science and Affiliated Societies at Pullman, Washington, June 16–17, 1932.

There was an average attendance of 28 members at the 3 half-day sessions of the society. Nineteen papers were read, abstracts of which follow the present report.

At the business meeting the following officers were elected to serve the society the next 2 years:

- President H. E. Morris, Montana State College, Bozeman, Montana.
- Vice President.....F. P. McWhorter, Oregon State College, Corvallis, Oregon.
- Councilor......J. M. RAEDER, University of Idaho, Moscow, Idaho.

Aside from the commendable harmony throughout our own meetings, the meetings of the entire Pacific Division, A. A. A. S., and Affiliated Societies were characterized this year by a delightful informality that contributed greatly to sustaining interest throughout the sessions. Dinners at which members of various biological societies met and fraternized were the order rather than social gatherings restricted by professional preferences. Such dinners, attended by people engaged in widely unrelated research, were the occasion of much merrymaking and pleasant foolery, especially the one given on the College Commons; the presidents of various societies were obliged to discuss, impromptu, some topic assigned them. Ours explained "What the Phytopathologists Have Contributed to the Depression," to the great amusement and satisfaction of all.

B. A. Rudolph, Secretary-Treasurer

ABSTRACTS

Perennial canker and anthracnose fungi: host relations and cultural differences.—
J. R. Kienholz. Gloeosporium perennans was observed naturally infecting quince trees and fruits, and the service berry (Amalanchier pallida) during 1931, while Neofabraea malicorticis was found on the native Oregon crab apple, Malus rivularis.

Plants successfully inoculated with both the perennial canker and anthracnose fungi include the yeach, service berry, wild cherry, plum, apricot, Oregon crab apple, flowering quince, cherry, hawthorn, mountain ash, and rose haws. Either the service berry or Oregon crab apple may have formed a native host plant for these organisms.

Dyes of the tri-phenyl-methane series inhibited spore germination at high dilutions. When malachite green was incorporated into either potato-dextrose or Coons' synthetic agar at a concentration of 1–200,000, the growth of 3 strains of Neofabraea malicorticis was inhibited from 20–90 per cent more than the growth of the 5 strains of Gloeosporium perennans used. Fairly accurate measurements gave surprisingly constant results, which give this method merit as a means of rapidly distinguishing these fungi by laboratory means. For constant results mycelial transfers from cultures previously transferred at least twice on a standard laboratory medium should be used, since spore material or very new cultures exhibit a higher degree of sensitiveness to the toxic action of the dyes.

The spotting of pineapple leaves caused by Pseudococcus brevipes (Ckl.), the pineapple mealybug.—W. Carter. Under field conditions in Hawaii two general types of spotting of pineapple leaves result from feeding by Pseudococcus brevipes. One appears as the typical chlorotic area following Coccid feeding. The other is designated as green spotting. This latter type is caused by the feeding of certain individuals and is not common to the species. Colonies have been maintained for a period of 1 year without any green spots resulting. Other colonies have consistently produced green spots. Another colony was apparently inherently capable of producing green spots, for these appeared at rare intervals.

Attempts at transmission of green spots, either by mechanical means or by transfer of nongreen-spotting mealybugs to healthy leaves after feeding on green spotted tissue, were all unsuccessful. Evidence that the ability to produce green spots is transmitted from the mature mealybug to her young is conclusive.

The green-spotting mealybug is frequently distinguishable from those unable to produce green spots by a difference in body color, a dark brown color of the body fluids that imparts a grayish cast to the waxy covering of the insect. The hereditary nature of the ability to produce green spots lead to a consideration of the possible relationship between the intracellular symbionts of the insect and its secretions.

The pineapple mealybug (Pseudococcus brevipes (Ckl.)) and wilt of pineapples.—
W. Carter. Mealybug wilt is common to all the pineapple-growing areas in Hawaii
and is the major known source of collapse of pineapple plants in the islands. The disease assumes many forms, dependent upon the size and time of onset of the initial mealybug population, the age, vigor, and succulence of the plant, and the fact that recovery
in various degrees is commonly encountered. The relationship between the pineapple
mealybug and wilt of pineapples has been conclusively demonstrated.

Quick wilt develops after a sudden attack by a fairly large colony of mealybugs, which may, however, feed for only a short time. Slow wilt is the result of a continuous attack by a gradually increasing population of mealybugs; its incidence is governed by the size of the mealybug colony and the length of time the colony feeds on the plant.

The disease is believed to be due to the secretion by the insect of a nonliving but toxic principle that is variably diffusible, sometimes affecting only the area of leaf actually fed upon by the insects, sometimes producing wilt in 2 or 3 contiguous leaves, or, as is usually encountered under field conditions, a wilted condition of the entire plant.

Comparison of quick and slow wilts suggests an antitoxic reaction by the plant,

which enables it to accommodate itself to large but, gradually developed, populations of the mealybug. The toxicity of *Pseudococcus brevipes* appears to vary with the kind and condition of the insect's host plant.

Observations on the ascospore discharge of pear-scab fungus, Venturia pyrina.—
L. Childs. Scab is one of the most important diseases affecting pears in parts of northwestern Oregon and western Washington. Since pear varieties grown in these areas exhibit great differences in their reaction to fungicides, general recommendations cannot be made for controlling scab. Lime-sulphur and Bordeaux sprays cannot be used with safety on tender-skin fruits, while various sulphur sprays including dry-mix, atomic sulphur, calcium mono-sulphide, and others have given indifferent results.

While recording ascospore discharge for *Venturia pyrina*, perithecia were noted in scabbed twig lesions. Although very similar to those of the scab organism, these perithecia were more superficial and apparently bore no setae. This associated organism is provisionally identified as *Mycosphaerella tulasnei*, having a Cladosporium imperfect form. Previous ascospore-discharge records for the scab parasite have been complicated by this second organism and hence dates of earliest spore expulsion may be inaccurate. However, both organisms discharge their spores from overwintered leaves during rain periods only, for at least as late as July. During 1932 the first Mycosphaerella spores were discharged on March 26. The rôle of this associated organism is unknown, having been previously reported only on cereal crops. Wherever it occurs on fallen leaves, scab perithecia are extremely rare or absent, even though scab lesions are present.

The fungicidal control of Phytophthora heart rot of pineapple plants.—F. P. MEHRLICH. Heart rot of the pineapple plant in Hawaii is caused by 4 described species of Phytophthora. Experiments in naturally infested areas involving 2 of these fungi have shown that Bordeaux 1–0.65–3 as a dip is an effective and economical preventive. This formula was selected from 67 formulae of 22 liquid fungicides and 12 dry fungicides tried. Bordeaux 1–0.65–3, applied by completely immersing planting material in it, has given better control than larger quantities of the same or different fungicides applied in other ways. A single application in 8 separate experiments has given the following control under conditions extremely favorable to development of the disease. Average control 79.23 per cent, range 63.40–91.59 per cent; disease in adjacent nontreated plots, average 48.8 per cent, range 22.70–84.99 per cent. The total concentration of Bordeaux 1–0.65–3 may be doubled or either constituent varied 50 per cent without injuring plants treated. The cost for materials is \$7.00 per acre of 10,000 plants. Specific recommendations for the use of this treatment have been made.

Indications of differential susceptibilities of various classes of planting materials have been presented.

Some experiments with mechanical transmission of the curly-top virus.—B. F. DANA. The need for a mechanical method of transferring the curly-top virus, naturally transmitted by the beet-leaf hopper, Eutettix tenellus Baker, has induced the writer to investigate this problem. Of the methods tried, a modification of the technique used by Sein in transmitting sugar-cane mosaic (Jour. Dept. Agr. Puerto Rico 14: 49-68. 1930) has been used most extensively. By this method, attempts have been made to transfer the disease from sugar beet to sugar beet, tomato, and spinach, and from tomato to sugar beet.

The first attempt to transfer the virus from sugar beet to sugar beet was successful in 8 out of 16 plants in 1 lot. A recently infected inoculum plant was used. Subsequent

trials have given low percentages of successful transfer. The percentage of successful transfer from sugar beet to tomato and spinach and from tomato to sugar beet has also been low.

It is apparent that this virus is most active soon after it is introduced into a plant and at this time is more amenable to mechanical transfer from plant to plant than at any subsequent time. The variability of the results obtained with this multiple-needle method indicates modifying factors that must be determined before the method can be used in routine work. This method has given 100 per cent transfer when used with a mosaic of cruciferous plants, which illustrates the variable in facility with which the different viruses may be transmitted by mechanical methods.

Narcissus-mosaic symptoms.—F. P. McWhorter. Narcissus-mosaic symptoms show great varietal variation and considerable variation among individuals of the same variety. Plant responses resulting from reduction in vigor occur in all varieties. The reduction in vigor leads to early maturity and renders infected plants especially susceptible to Stagonospora leaf spot and Ramularia blight. Symptoms accountable to chlorophyll repression include yellow striping, mottling, marbling and, occasionally, yellowing of entire leaves. These chlorophyll disturbances can be analyzed by photographing the leaves between thick, greenish glass plates, using daylight, an A "red" filter and panchromatic plates. The leaf surfaces of some varieties become roughened, apparently, as a result of the disease. The leaves of some varieties curl; those of others bend in the plane of the leaf. Shortly after blooming, necrotic areas may develop in epidermal tissues of the shoot and exhibit a bronzing effect. The perianth lobes of some varieties show hyaline streaks. The disease is bulb-perpetuated and the symptoms of each individual remain reasonably constant from year to year.

A preliminary analysis of tulip breaking.—F. P. McWhorter. The theory is advanced that the usual variegated flower-color condition of tulips, commonly called "breaking" and technically referred to as "mosaic," results from the action or interaction of two viruses. One of these viruses carries a color-adding factor and produces no visible effect on leaves; the other removes flower color and strongly stripes the leaves.

It was observed that certain varieties, when broken, tend to segregate into plants or parts of plants (clumps) bearing strongly darkened flowers or decidedly bleached flowers. By encouraging this segregation through selection and by using inocula prepared from the parts of flowers where the color was removed and darkened, respectively, it has been possible to secure the viruses in almost pure condition. The color-adding virus has little effect on plant growth and may be of practical use in producing 'new' varieties. The color-removing virus is extremely virulent and reduces plant growth to formal. Cross inoculations with the juice from leaves of mosaic-diseased speciosum lily indicate that these contain a virus indistinguishable from, if not identical with, the color-removing virus of tulips. The relationship of the color-adding virus to other plant viruses has not been determined.

A new method of inoculating with viruses.—L. K. Jones. A method of inoculating plants with viruses has been developed that has proved very effective in transmitting the latent and veinbanding viruses of potato as well as the virus of tobacco mosaic. With this method the worker can inoculate a larger population in a given time with a minimum of preparation of equipment. The possibility of contamination is almost eliminated.

Round swab sticks or "applicators," as used by the medical profession, are broken in half. Absorbent cotton is firmly rolled onto one end of each half applicator and securely fastened with thread. These swabs are placed in a cannister with the cotton ends down and sterilized in the autoclave at 20 pounds' pressure for 1 hour. Six-inch pot labels are placed in a cannister with the pointed ends up and sterilized.

In making inoculations from growing plants, portions of the plant tissue are broken off with the sterilized swab and pot label. The plant tissue is macerated on the label, then rubbed over the foliage to be inoculated with the swab. The pot label acts as a support of the leaf while being inoculated. By using care, neither the tissue used as inoculum nor the plant to be inoculated comes in contact with any source of contamination.

Dried host tissue may be used in this method by macerating the tissue in a small amount of water in a sterilized Petri dish with the aid of the sterilized swab and label.

The sources of the viruses that cause streak of tomato.—L. K. Jones. Streak of tomatoes as found in the greenhouses of the State of Washington is caused by a combination of the latent potato virus and the common tobacco-mosaic virus. Once the disease appears on plants in the greenhouse it is spread very rapidly by pruning and cultural practices.

The tobacco-mosaic virus appears to be introduced in the greenhouses, mainly, by the use of tobacco by the workmen, although it may sometimes be transmitted to tomatoes from petunias, *Solanum nigrum*, or other host plants found in the greenhouse.

It has been shown that the potato-latent virus can be brought into the greenhouse and transmitted to tomatoes in two ways. First, from volunteer potato plants in the tomato-plant bed, which is due to the use of soil that has previously grown potatoes. Forced contact of the potato and tomato foliage will transmit the latent virus. Second, workmen handling potatoes, in sorting or removing sprouts, can transmit the latent virus if tomato plants are handled, following work with the potato tubers.

Aspergillus sclerotiorum, n. sp., and its relation to decay of apples.—G. A. Huber. A new species of Aspergillus is described as belonging to the Aspergillus ochraceus group, sulphureus series. This organism, for which the name A. sclerotiorum is suggested, was found to be pathogenic on apples, causing decay both at ordinary and at cold-storage temperatures. When inoculated into sound, ripe Jonathan apples, it produced lesions 42-46 mm. in diameter in 42 days at 22°-25° C. It produced lesions 28-38 mm. in diameter in 90 days at 6°-8° C. and lesions 10-14 mm. in diameter in 120 days at 0°-2° C.

The influence of moisture on the development of the Cercosporella foot rot of winter cereals.—RODERICK Sprague. Cercosporella herpotrichoides From, the cause of a destructive foot rot of winter cereals in the Columbia Basin of Washington and Oregon, occurs in semiarid prairie regions where the annual rainfall ranges from 14 to 24 inches. Its optimum development occurs in those parts having just under 20 inches a year.

A warm, moist March, followed by an equally moist, cool April and early May, brings on severe infection, especially when the plants have been forced by prolonged growth the preceding autumn.

Foot rot thrives in fine sandy-loam soil during the time of year when this soil has abundant moisture. When the dry season begins, usually in early summer, the surface-soil moisture is quickly reduced to almost oven-dry condition and foot rot almost ceases to develop. All experimental evidence and field observations show that soil moisture,

which is directly correlated with seasonal precipitation, is the determining factor in the relative severity of the disease in different years.

(Cooperative investigations between the Division of Cereal Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture, and the Oregon and Washington agricultural experiment stations.)

The importance of lenticel infection of apples by Penicillium expansum.—K. F. Baker and F. D. Heald. Blue mold (Penicillium expansum) of apples is generally considered as essentially a wound parasite, although infection through the lenticels has been reported. Stricter grading and improvements in packing equipment since 1925 would indicate a decrease in the amount of injured fruit in packed boxes. This is not consistent with the fact that the annual loss from blue mold has remained fairly constant in those years.

Examination of apples in commercial storage in Yakima and Wenatchee, Washington, has shown that infection by blue mold through the lenticels frequently occurs. The maximum amount of such infection found in any lot was 33.3 per cent; incidence up to 10 per cent is fairly common. Two or 3 such infections per apple are not unusual, and up to 14 have been observed. Lenticel infection has been observed in 8 apple varieties. Splitting of the skin of the decayed areas and the emergence of the fungus have made difficult the determination of the infection court.

Lenticel infection may be responsible for a considerable portion of the average annual loss from blue mold decay, and it, rather than mechanical injury, is the cause of many of the lots showing high percentages of blue mold in storage.

Walnut blight and its control in Oregon.—P. W. MILLER. Studies on walnut blight and its control carried on in Oregon during the past 2 years show (1) that the causal organism, Ps. juglandis, overwinters in this State primarily in infected buds; (2) that meteoric water is the most important agency concerned in the spread of primary and secondary infections; and (3) that the use of home-made Bordeaux mixture is an effective means of controlling walnut blight where a suitable schedule of applications is used during the critical period for infection. In tests carried on in 1930 and 1931, respectively, Bordeaux mixture 3-3-50 seemed to be practically as effective as greater strengths. At least two applications of Bordeaux appeared to be necessary to control blight satisfactorily in grafted orchards. Best results from spraying were obtained when the applications were made (1) just before blooming of the pistillate flowers and (2) just after blooming, when the stigmas of the pistils were turning brown. Under severe epidemic conditions a third treatment applied about 2 weeks after the second application appeared to be necessary to control blight. Dusting has not given sufficiently good control thus far to recommend its use. Injury to set of nuts occurred from spraying with Bordeaux when the pistillate flowers were fully open to receive pollen. Bordeaux injury of young foliage was observed but it did not appear to be extensive enough to appreciably damage the trees or affect the crop unfavorably. Altering the method of preparing Bordeaux did not eliminate this foliage injury. Spraying with Bordeaux appears to have resulted in a profit to the grower through increased yield and quality of nuts. Indirect benefits through reduced hold-over infections are indicated.

Mosaic disease of horse-radish.—B. F. Dana and F. P. McWhorter. Horse-radish plantings in Washington County, Oregon, deteriorated during the 1930-1931 season. At digging time most of the plantings were dwarfed and yellowed. Roots averaged small

and were unsightly because of rough scaly surfaces and unsalable because of pithy texture and frequent dark streaks.

Root cuttings from plants showing various stages of decline were potted and forced at different temperatures. The foliage was more or less stunted and leaf blades were strikingly segmented in a fern-like manner. The young leaves exhibited a prominent mosaic-like mottle, characterized by interveinal pale green areas interspersed with dark green. Clearing of veins was not noticeable. Old leaves developed black elongated lesions in the epidermis and outer cortex of the petioles.

Cross inoculations to turnip and mustard were made with the Oregon material by means of a modification of the Sein multiple-needle method (Jour. Dept. Agr. Puerto Rico 14: 49-68. 1930). One hundred per cent of the plants inoculated developed a mosaic similar to that on the horse-radish. Symptoms were apparent within 10 to 12 days, plants became dwarfed, and many died within a short time.

This preliminary work indicates that a serious mosaic is partly responsible for the reported deterioration of horse-radish.

The sources of contamination of the normal apple and spore load.—G. A. Huber and F. D. Heald. The first contamination of normal apples occurs in the orchard. Analyses of the air in various orchards at harvest time showed from 25 to approximately 500 spores of fungi per cubic foot. Apples carefully picked from the trees and so handled as to prevent further contamination showed from 14,000 to 159,100 spores per apple. Apples from surface-irrigated plots showed an average of 36,766 spores per apple, while those from overhead-irrigated plots showed an average of 119,616 spores.

"Dirty" picking boxes, important sources of contamination of normal apples, showed an average load of 108,050,160 spores of fungi on the interior surface. A box with the maximum contamination carried a load of 109,958,400 spores on the inner surface of the bottom boards only, of which 32,987,520 were Penicillium types, the majority being *P. expansum*.

The air in packing houses during the packing season showed from 32 to 994 spores per cubic foot, fewer spores occurring in houses where sanitary measures were practiced. Various processes used for the removal of the arsenical residue from apples did not reduce the spore load of normal apples to any extent in 1926. In 1927, the improved methods reduced the spore load to a certain extent but not enough to have an appreciable effect in the prevention of storage rots. In 1930, the methods used, namely, acid, Laux, and Brogdit-Brogdex, greatly reduced the spore load of the apples, but neither acid nor alkali processes had noticeable effect on the types of fungi present. Gloves worn by sorters showed from 12,100 to 40,000 spores per square inch in the palms.

Physiology and pathogenicity of species of Phytophthora that cause heart rot of pineapple plants.—F. P. Mehrlich. Heart rot of the pineapple plant in Hawaii is caused by Phytophthora cinnamomi Rands, Ph. meadii McRae, Ph. melongenae Sawada, and Ph. parasitica Dastur. An extended geographic range of the disease is given. Newly found hosts of these fungi include common weeds and green-manure plants, which may aid their survival in the absence of pineapple plants. Three of these fungi may also rot green pineapple fruits. Symptoms and causal organisms relate pineapple heart rot to coconut bud rot.

Detailed evidence is presented that *Pseudopythium phytophthoron* Sideris is a synonym of *Phytophthora cinnamomi* Rands. It occurs in virgin soil associated with *Dicranopteris emarginata* (Brack) Robinson. Cultures isolated locally have been compared

with authentic cultures of Rand's isolations and found similar to them in host range, temperature relationships, physiology of conidial production, morphology of mycelium, and reproductive structures. This organism may rot green fruit and cause root rot in addition to heart rot.

A method for isolating these fungi from soil is described. It consists in incubating susceptible leaf tissue in a mixture of infested soil and sterile water. Pure cultures are isolated from the tissue by planting or by inoculating pineapple crowns and subsequently planting them. The method has been used for a variety of purposes.

Enzumes with Cladosporium.—H. CAMPBELL.

The longevity of the latent and veinbanding viruses of potato in dried leaf tissue.—
B. Burnett.

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Koppers Flotation Sulphur is an improved, mild fur gicide. Controls Apple Scab, Peach Brown Rot, Cherry Leaf Spot and other fungous diseases. It has the following advantages:

- 1. All sulphur particles uniformly microscopic-90% of particles are less than 3/25000-inch across.
- 2. Quickly mixes with water and remains in suspension with the slightest agitation. Does not settle out to form a hard cementlike mass as ordinary wettable sulphurs do.
- 3. It is not caustic which makes it especially popular with the men who actually do the spraying.
- 4. Dependable uniformity—prepared under strict laboratory control.

- 5. Dependable protection—it has had five years' testing in the field under the supervision of the Crop Protection Institute.
- 6. Does not harden in stationary or other spray equipment.
- 7. Manufactured and guaranteed by a reliable concern.

Your cooperation is invited in testing these sulphurs in your territory. Extensive field data are available for many sections of the country. Koppers Flotation Sulphur is the only type of sulphur originating from illuminating gas which is endorsed by the Crop Protection Institute and state experiment stations.

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